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(54) Title: HYPOCHOLESTEROLEMIC AGENTS (57) Abstract This invention relates to certain steroidal glycosides useful as hypocholesterolemic agents and antiatherosclerosis agents and certain protected intermediates useful in the preparation of said steroidal glycosides.		

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HYPOCHOLESTEROLEMIC AGENTS

Background of the Invention

This invention relates to steroidal glycosides and methods of using the same, particularly as hypocholesterolemic agents and antiatherosclerosis agents, in mammals.

Many known products possessing hypocholesterolemic activity are cross-linked synthetic polymer derivatives. For example, cross-linked, water-insoluble, bile-acid-binding polystyrene-based resins, e.g., Cholestyramine® agents, have a gritty "mouth-feel", and thus have poor palatability. In addition, these resin beads typically have a low in vivo efficiency. Thus, the effective hypocholesterolemic dose of these materials is excessive, typically 18-24 grams of formulated product per day. Other known polymers having hypocholesterolemic activity include the natural product chitosan and chitosan derivatives as described in European Application pub. no. 0212145. However, the effective hypocholesterolemic dose of these materials is also high.

Other known hypercholesterolemia controlling agents include plant extracts such as "alfalfa saponins". However, these plant extracts are of variable composition and contain significant amounts of non-useful chemical substances. Due to the variations in composition, it is difficult to set a standard dosage or predict the impurities present. Thus, such extracts are not well suited for use by humans. Furthermore purification of these extracts would be expensive. As an alternative certain synthetically produced, pure "sapogenin-derived" compounds e.g., substances compounded from spirostane, spirostene or sterol-derived compounds depress cholesterol absorption more effectively than alfalfa extracts on a weight basis and thus can be administered in reasonable sized doses. Because the chemical compositions of these substances are known and because they can be synthesized at a high degree of purity, they are suitable for use by any warm-blooded animal, including humans.

However, unless administered in massive amounts, pure sapogenins do not significantly inhibit cholesterol's absorption. It is only when compounded with another moiety that sapogenins have the desired effect. Examples of such sapogenin compounds are compounds of tigogenin and diosgenin, particularly glycosides thereof. P.K. Kintia, Iu. K. Vasilenko, G.M. Gorianu, V.A. Bobeiko, I.V.

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Suetina, N.E. Mashchenko, Kim. Pharm. Zh., 1981, 15(9), 55 discloses 3-O-(β -D-galactopyranosyl)hecogenin and its use as a hypocholesterolemic agent. U.S. Pat. Nos. 4,602,003 and 4,602,005 disclose certain steroidal glycosides, in particular 3-O-(β -D-glucopyranosyl)tigogenin and 3-O-(β -D-cellobiosyl)tigogenin and their use for the control of hypercholesterolemia. 3-O-(β -D-cellobiosyl)tigogenin has superior hypocholesterolemic activity when compared to, for example, cholestyramine. PCT publication WO 93/07167 discloses several steroidal glycosides in particular 3-O-(5-C-hydroxymethyl-L-arabino-hexopyranosyl)-tigogenin and 3-O-(5-C-hydroxymethyl-L-arabino-hexopyranosyl)-diosgenin and their use in the control of hypercholesterolemia.

Recently commonly assigned PCT publication WO 93/11150 has disclosed a number of steroidal glycosides including 11-ketotigogenin- β -O-cellobioside, hecogenin- β -O-cellobioside, diosgenin- β -O-cellobioside and their use as antihypercholesterolemic agents. Also commonly assigned PCT publication WO 94/00480, the disclosure of which is hereby incorporated by reference, discloses a variety of steroidal glycosides and their use as antihypercholesterolemic agents.

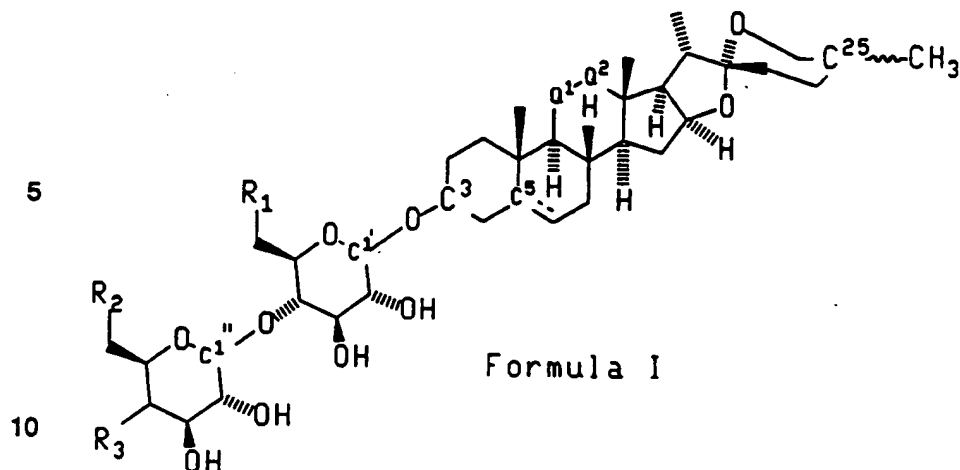
Although the hypocholesterolemic compounds described above make a significant contribution to the art there is a continuing search in this field of art for improved hypocholesterolemic pharmaceuticals.

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Summary of the Invention

This invention is directed to steroidal glycosides, particularly spirostanyl glycosides, that are useful as hypocholesterolemic agents and antiatherosclerosis agents. The compounds of this invention have the formula

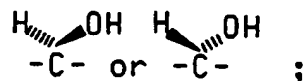
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and the pharmaceutically-acceptable salts and hydrates thereof

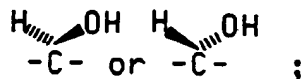
wherein

15 Q¹ is carbonyl,



Q² is methylene, carbonyl,

20



R₁, R₂, and R₃ are each independently hydrogen, hydroxy, halo, amino, azido, (C₁-C₆)alkoxy(C₁-C₆)alkoxy or -Z-R₄;

25 Z for each occurrence is independently -NH-C(=O)-, -O-C(=O)-, -O-C(=O)-N(R₅)-, -NH-C(=O)-N(R⁵)- or -O-C(=S)-N(R⁵)-;

R₄ for each occurrence is independently aryl, aryl(C₁-C₆)alkyl, (C₂-C₄)alkenyl, (C₁-C₆)alkyl, cyclo(C₃-C₇)alkyl or cyclo(C₃-C₇)alkyl(C₁-C₆)alkyl; each R₄ optionally mono-, di-, or tri-substituted independently with halo, (C₁-C₄)alkyl, hydroxy, phenoxy, trifluoromethyl, nitro, (C₁-C₄)alkoxy, methylenedioxy, oxo, (C₁-C₄)alkylsulfanyl, (C₁-C₄)alkylsulfinyl, (C₁-C₄)alkylsulfonyl, dimethylamino, mono- or di-(C₁-C₄)alkylaminocarbonyl, (C₁-C₄)alkylcarbonyl, (C₁-C₄)alkoxycarbonyl, pyrrolidinylcarbonyl wherein aryl is carbon-linked and is phenyl, furyl, thienyl,

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pyrr lyl, xaz lyl, isoxaz yl, oxadiazolyl, thiazolyl, isothiaz lyl, b nzo thiazolyl, thiadiazolyl, pyrazolyl, imidazolyl or pyridyl; and

- R_5 for each occurrence is independently hydrogen, (C_1-C_4) alkyl or R_5 is such that when taken together with the nitrogen to which it is attached and with R_4 ,
 5 wherein R_4 is a covalent bond, it forms pyrrolidiny, piperidiny, N-methylpiperaziny, indoliny or morpholiny and such cyclic group may be substituted on carbon with (C_1-C_4) alkoxycarbonyl;

with the proviso that R_1 , R_2 and R_3 are not all hydroxy.

- A first group of preferred compounds of Formula I consists of those
 10 compounds wherein R_1 , R_2 and R_3 are each independently hydroxy or $-Z-R_4$, Z is $-O-C(=O)-N(R_5)-$ and R_5 is hydrogen. Within this first group of preferred compounds are especially preferred compounds wherein the $C^{1'}$ anomeric oxy is beta, the $C^{1''}$ anomeric oxy is beta, R_3 is alpha, the C^5 hydrogen is alpha, C^{25} is (R), the C^3 oxy is beta, Q^1 is carbonyl, Q^2 is methylene and R_1 is hydroxy. Particularly preferred
 15 compounds within the above group of especially preferred compounds are compounds wherein R_2 and R_3 are $-Z-R_4$ and R_4 is 2-fluorophenyl, 2,4-difluorophenyl, 2-methylphenyl, 2-thienyl-methyl, 2-methoxycarbonyl-ethyl, thiazol-2-yl-methyl, 2-methoxycarbonyl-butyl or phenyl. Other particularly preferred compounds within the above group of especially preferred compounds are compounds wherein R_2 is $-Z-R_4$,
 20 R_3 is hydroxy and R_4 is 2,4-difluorophenyl, 2,6-dichlorophenyl or 2-fluorophenyl.

Other especially preferred compounds within the above first group of preferred compounds of Formula I are compounds wherein the $C^{1'}$ anomeric oxy is beta, the $C^{1''}$ anomeric oxy is beta, R_3 is alpha, the C^5 hydrogen is alpha, C^{25} is (R),



- the C^3 oxy is beta, Q^1 is carbonyl, Q^2 is $-\text{C}-$ and R_1 is hydroxy. Particularly
 25 preferred compounds within this group are compounds wherein R_2 and R_3 are $-Z-R_4$ and R_4 is 2-fluorophenyl, 2-thienyl-methyl, 2-methoxycarbonyl-ethyl or thiazol-2-yl-methyl.

- A second group of preferred compounds of Formula I consists of those compounds wherein Q^1 is carbonyl, R_1 is hydroxy, hydrogen, halo, azido, or (C_1-C_6) alkoxy
 30 C_6 alkoxy (C_1-C_6) alkoxy, R_2 is hydrogen, halo, azido, or (C_1-C_6) alkoxy (C_1-C_6) alkoxy, R_3 is $-Z-R_4$, Z is $-O-C(=O)N(R_5)-$ and R_5 is hydrogen. Within this second group of

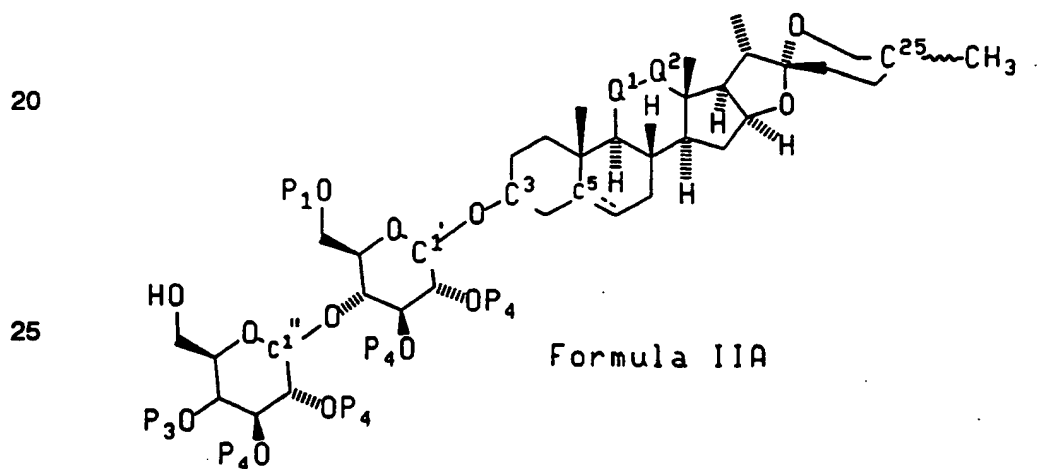
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preferred compounds are specially preferred compounds wherein the C^{1'} anomeric oxygen is beta, the C^{1''} anomeric oxygen is beta, R₃ is alpha, the C⁵ hydrogen is alpha, C²⁵ is (R) and the C³ oxygen is beta. Particularly preferred compounds within the above group of especially preferred compounds are compounds wherein R₁ is hydroxy, R₂ is hydrogen and R₄ is 2-fluorophenyl.

A third group of preferred compounds of Formula I consists of those compounds wherein R₁, R₂ and R₃ are each independently hydroxy or -Z-R₄, Z is -O-C(=O)-, R₄ is independently (C₁-C₆)alkyl, aryl or aryl mono- or disubstituted with halo or (C₁-C₆)alkyl. Within this third group of preferred compounds are especially preferred compounds wherein R₁ and R₂ are -Z-R₄, R₃ is alpha hydroxy, R₄ is 2-furyl, the C^{1'} anomeric oxygen is beta, the C^{1''} anomeric oxygen is beta, the C⁵ hydrogen is alpha, C²⁵ is (R), the C³ oxygen is beta, Q¹ is carbonyl and Q² is methylene.

A fourth group of preferred compounds of Formula I consists of those compounds wherein R₁, R₂ and R₃ are each independently hydroxy or halo, Q¹ is carbonyl, the C^{1''} anomeric oxygen is beta and R₄ is alpha.

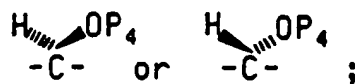
Protected intermediates of the above Formula I compounds include compounds of Formula IIA



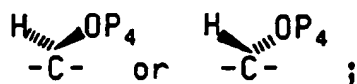
30 wherein

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Q¹ is carbonyl,



5 Q² is methylene, carbonyl,



P₄ is an alcohol protecting group; and

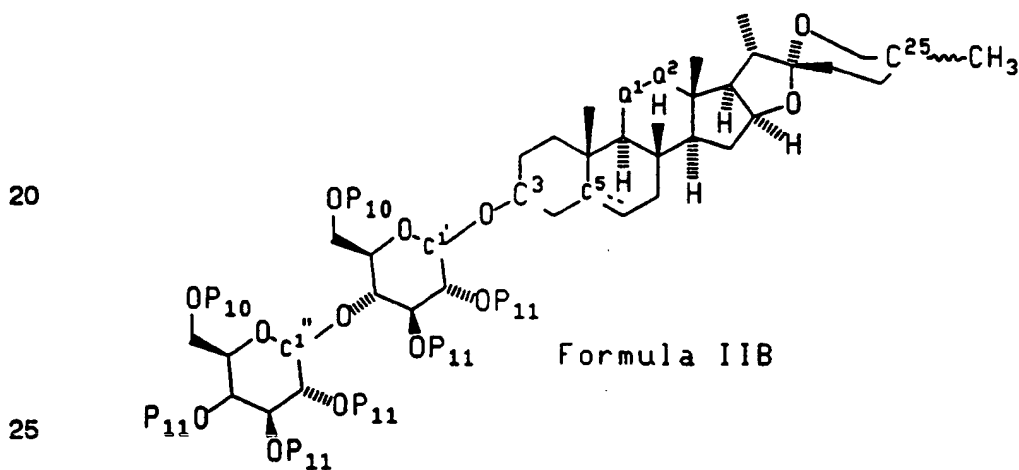
10 P₁ is hydrogen and P₃ is an alcohol protecting group or

P₃ is hydrogen and P₁ is an alcohol protecting group.

Preferred compounds of Formula IIA consists of these compounds wherein the alcohol protecting group is acetyl or chloroacetyl.

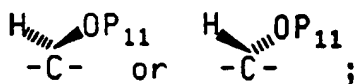
Other protected intermediates of the above Formula I compounds include

15 compounds of Formula IIB



wherein

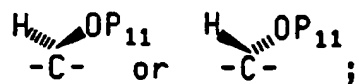
Q¹ is carbonyl,



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Q^2 is methyl ne, carbonyl,

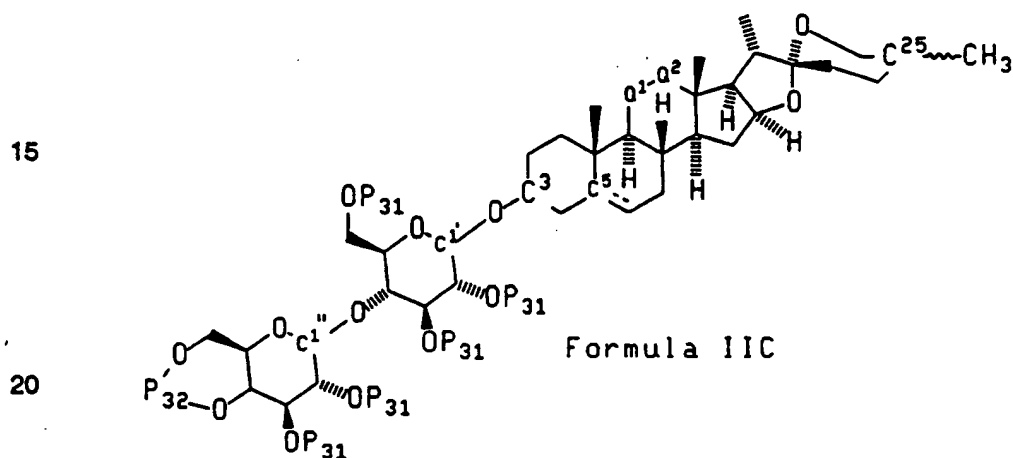


5 P_{10} is a silyl protecting group; and

P_{11} is an alcohol protecting group that is different from P_{10} .

Preferred compounds of Formula IIB consist of these compounds wherein the alcohol protecting group is acetyl and the silyl protecting group is t-butylidiphenylsilyl, triisopropylsilyl or t-butyldimethylsilyl.

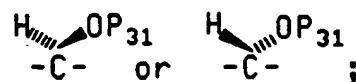
10 Yet still other protected intermediates of the above Formula I compounds include compounds of Formula IIC



wherein

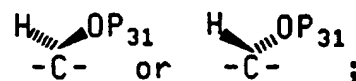
Q^1 is carbonyl,

25



Q^2 is methylene, carbonyl,

30



P_{31} is an alcohol protecting group; and

P_{32} forms a cyclic protecting group for a 1,3 diol.

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Preferred compounds of Formula IIC consists of those compounds wherein P_{31} is acetyl or chloroacetyl and P_{32} is benzylidene or paramethoxybenzylidene.

Yet another aspect of this invention is directed to a method for treating hypercholesterolemia or atherosclerosis in a mammal by administering to a mammal
5 suffering from hypercholesterolemia or atherosclerosis a hypercholesterolemia or atherosclerosis treating amount of a Formula I compound.

This invention is also directed to pharmaceutical compositions for the treatment of hypercholesterolemia or atherosclerosis in mammals which comprise a therapeutically effective amount of a compound of the Formula I and a
10 pharmaceutically acceptable carrier.

The compounds of Formula I are herein defined as the single enantiomer having the absolute stereochemistry depicted in Formula I.

By alcohol protecting group is meant a conventional alcohol protecting group known to those skilled in the art. Such alcohol protecting groups are described in
15 T. W. Greene, Protective Groups in Organic Synthesis, John Wiley & Sons, New York, New York, 1991, 2nd Edition, which is hereby incorporated by reference (e.g., see pages 10-13) and include for example, esters such as formyl, (C_1-C_{10}) alkanoyl optionally mono-, di- or tri-substituted with (C_1-C_6) alkoxy, halo, aryl, aryloxy or haloaryloxy; aroyl optionally mono-, di- or tri-substituted on carbon with halo, (C_1-C_6) alkyl, (C_1-C_6) alkoxy wherein aryl is phenyl, 2-furyl etc; carbonates; sulfonates;
20 and ethers such as benzyl, paramethoxybenzyl, methoxymethyl etc.

By silyl protecting group is meant a conventional trisubstituted silyl protecting group known to those skilled in the art such as is used to protect a hydroxy group (not to protect a silyl group). Such silyl protecting groups are described in the
25 above cited T. W. Greene book (e.g., page 12) and include for example, silyl compounds where each of the three silyl substituents may be (C_1-C_6) alkyl optionally substituted with (C_1-C_6) alkoxy, halo or aryl; and aryl optionally substituted on carbon with halo, (C_1-C_6) alkyl, (C_1-C_6) alkoxy wherein aryl is phenyl, 2-furyl etc.

By forms a cyclic protecting group for a 1,3 diol is meant a conventional
30 ketal or acetal protecting group known to those skilled in the art. Such cyclic protecting groups are described in the above cited T. W. Greene book (e.g., page 13 and 14) and include for example, wherein the protecting group is cyclic acetal, (C_1-C_6) alkyliden optionally substituted with (C_1-C_6) alkoxy or halo; and arylidene

optionally substituted on carbon with halo, (C₁-C₆)alkyl, (C₁-C₆)alkoxy wherein aryliden is phenylidene, 2-furylidene etc. and their cyclic ketal analogs wherein the additional substituent is (C₁-C₆)alkyl optionally substituted with (C₁-C₆)alkoxy or halo or aryl optionally substituted on carbon with halo, (C₁-C₆)alkyl or (C₁-C₆)alkoxy

5 wherein aryl is phenyl, 2-furyl etc.

By halo is meant chloro, bromo, iodo, or fluoro.

By alkyl is meant straight chain or branched saturated hydrocarbon.

This invention describes steroidal glycosides in which the sugars are substituted (e.g., with carbamoyl, thiocarbamoyl acyl and silyl groups). In the
10 nomenclature (see Examples and Preparations) all such groups are herein defined as substituted on oxygen unless otherwise designated as deoxy.

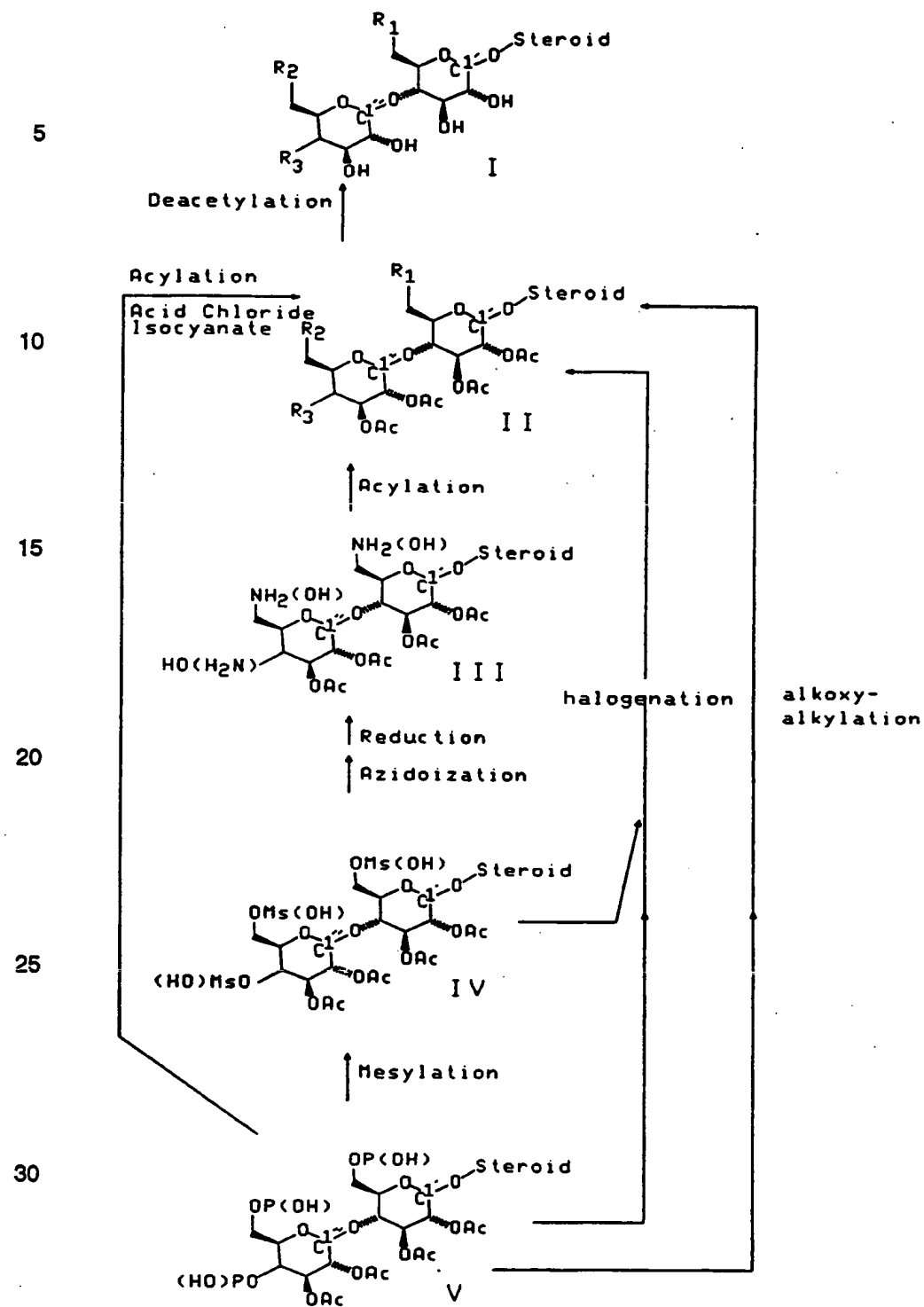
The Z moieties described above are herein defined such that they are to be read from left to right (i.e., the left or first atom is attached to the sugar molecule and not to R₄).

15 The C⁵-C⁶ dotted line in the above steroidal moiety is herein defined as an optional carbon-carbon double bond.

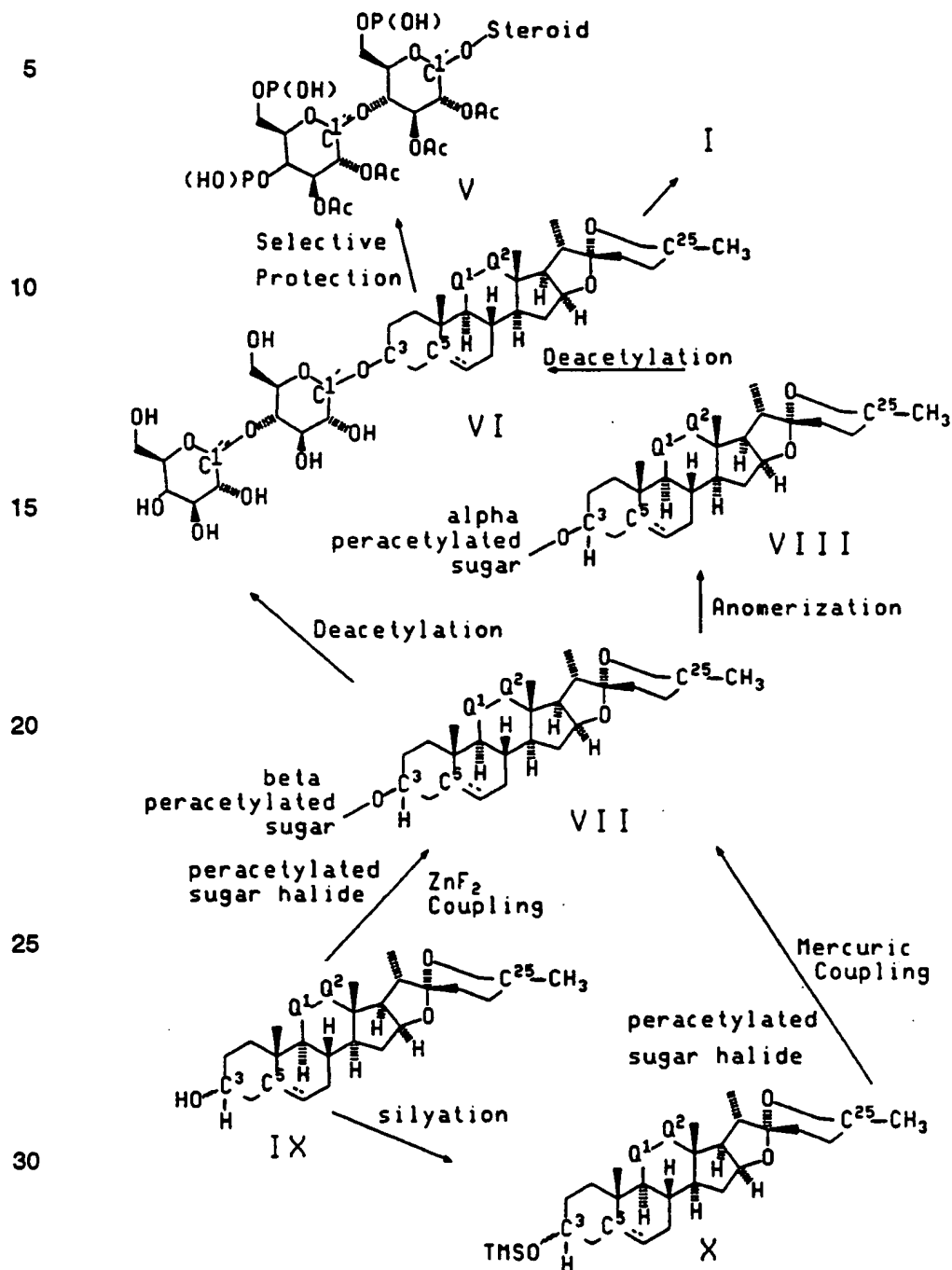
Other features and advantages will be apparent from the specification and claims which describe the invention.

Detailed Description of the Invention

SCHEME I

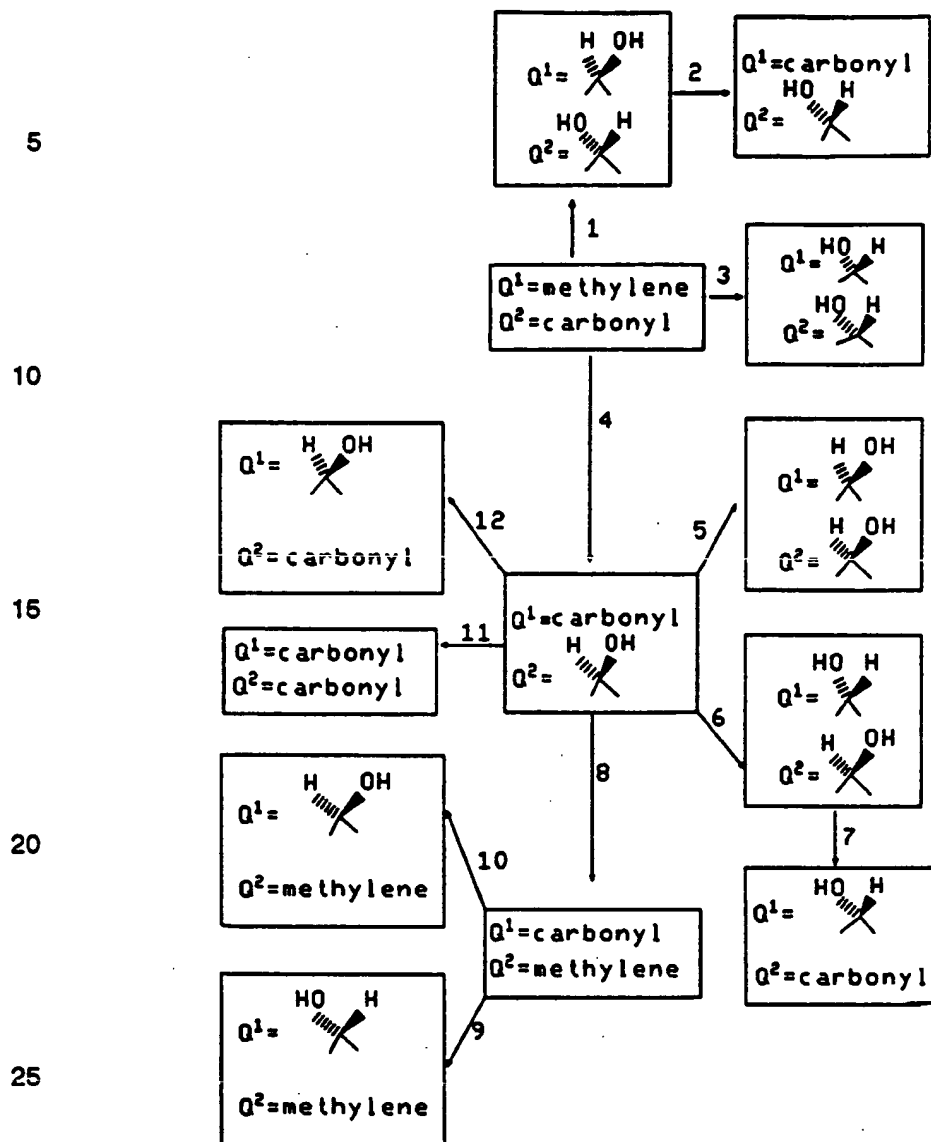


SCHEME II



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SCHEME III



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In general the compounds of this invention may be made by coupling the desired protected sugar halide and steroid followed by deprotection. The desired functionality/substituents are attached (following optional selective protection) and a final deprotection is performed. The following text (which is keyed to the above

5 Schemes) provides a more detailed description.

According to reaction Scheme I, the desired Formula I compounds wherein Steroid is the steroidal moiety of the Formula I compound shown above (i.e., wherein Q¹, Q², C³, C⁵, C²⁵ are as defined above) and C^{1'}, C^{1''}, R₁, R₂ and R₃ are as defined above may be prepared by deprotecting (e.g., deacetylating) the appropriate

10 Formula II compound wherein Steroid is the steroidal moiety described above, (although hereinafter in the Detailed Description those skilled in the art will realize that in those instances wherein Q¹ and/or Q² is hydroxy the hydroxy may exist in a conventionally protected form as a result of protection of the sugar), C^{1'} and C^{1''} as defined above and R₁, R₂, and R₃ are as defined above or each independently is

15 a conventionally protected hydroxyl group such as -OAc.

Typically the deprotection (preferably the deacetylation), is accomplished by combining the Formula II compound with a nucleophilic base such as sodium methoxide or potassium cyanide in a polar solvent such as methanol, tetrahydrofuran, n-propanol or mixtures thereof at temperatures of about 0°C to

20 about 100 °C (typically at ambient temperatures) and pressures of about 0.5 psi to about 50 psi (typically ambient) for about 0.25 hour to about 2 hours.

Additionally, the compounds may contain a silyl protecting group which can be removed by treating the deacetylated product from above with a quaternary ammonium fluoride such as tetrabutyl ammonium fluoride in an anhydrous solvent

25 such as tetrahydrofuran at temperatures of about 0°C to about 50°C (typically at ambient temperatures) for about 0.1 to about 3 hours.

The desired Formula II compounds wherein Steroid is the steroid moiety described above and C^{1'} and C^{1''} are as defined above and at least one of R₁, R₂ and R₃ is hydrogen can be prepared by reduction of the corresponding halogenated

30 compound. Typically, the reduction can be performed by treating the halogenated compound (Br and I preferred) with a reducing agent such as tri-n-butyl tin hydride and a radical initiator such as azoisobutyronitrile (AIBN) in an anhydrous aprotic solvent such as toluene at reflux temperature for about 1 hour to about 5 hours.

The desired Formula II compounds wherein Steroid is the steroidal moiety described above, C^{1'} and C^{1''} are as described above and at least one of R₁, R₂ and R₃ is halogen may be prepared by halogenation of the appropriately protected Formula V compound wherein Steroid is the steroidal moiety described above, C^{1'} and C^{1''} are as defined above and R₁, R₂, and R₃ are each independently hydroxy or a conventionally protected hydroxyl group such as -OAc.

Generally the halogenation can be performed by first preparing an appropriately activated and protected form of the Formula V compound (e.g., the Formula IV mesylate) followed by treatment with the desired lithium halide. Typically the mesylation can be performed by combining the Formula V compound and mesyl chloride in the presence of a base, preferably an amine base such as triethylamine and a catalytic amount of a catalyst such as dimethylaminopyridine in an aprotic, anhydrous solvent such as anhydrous dichloromethane at a temperature of about -20 °C to about 20 °C for about one hour to about four hours. The resulting mesylate is then treated with the appropriate lithium halide in a polar solvent such as N, N-dimethylformamide at a temperature of about 70 °C to about 100 °C for about one to about three hours.

Alternatively, the iodination can be performed by combining iodine and the appropriate Formula V compound in an anhydrous aprotic solvent such as toluene (in the presence of imidazole and triphenylphosphine) under reflux conditions and ambient pressure for about four to about eight hours.

Alternatively, the fluorination can be performed by combining the appropriate Formula V compounds with a fluorinating agent such as dialkylaminosulfur trifluoride (e.g., DAST) in an anhydrous, aprotic solvent such as dimethoxy ethane or dichloroethane at a temperature of about -10 °C to about 10 °C and then after about twenty minutes to about two hours raising the temperature to about 30 °C to about 60 °C for about one hour to about four hours.

Alternatively, a selective bromination (i.e., R₂=Br) can be accomplished by treating the appropriate Formula V compound (wherein C^{6'} and C^{6''} are substituted with OH and C^{6'} is substituted with a conventionally protected hydroxyl group such as -OAc) with carbon tetrabromide and triphenyl phosphine and an amine base such as pyridine in an anhydrous aprotic solvent such as dichloromethane at ambient temperature for about 6 hours to about 48 hours.

The desired Formula II compounds wherein Steroid is the steroidal moiety described above, C^{1'} and C^{1''} are as described above and at least one of R₁, R₂ and R₃ is (C₁-C₈)alkoxy(C₁-C₈)alkoxy may be prepared by alkylating the appropriately protected Formula V compound wherein Steroid is the steroidal moiety described
 5 above, C^{1'} and C^{1''} are as defined above and C^{6'}, C^{6''} and C^{4''} are each independently substituted with hydroxy or a conventionally protected hydroxyl group such as -OAc.

Typically, the appropriate Formula V compound is combined with an excess of the appropriate alkoxyalkyl halide and a trialkyl amine base such as
 10 diisopropylethylamine in the presence of an anhydrous, aprotic solvent such as dichloroethane at a temperature of about 15 °C to about 35 °C (typically ambient temperature) for about one to about eight hours followed by mixing for one to four hours at a temperature of about 40 °C to about 70 °C.

The desired Formula II compounds wherein Steroid is the steroid moiety
 15 described above and C^{1'} and C^{1''} are as defined above and at least one of R₁, R₂, and R₃ is Z-R₄ and R₄ contains a ketone group can be prepared by oxidation of the corresponding hydroxy substituted Formula II compound. Typically the oxidation is performed by treating the hydroxy compound with an oxidizing agent, such as pyridinium chlorochromate, in an anhydrous halogenated solvent such as
 20 dichloromethane at 0°C to about 30°C, generally at ambient temperatures, for about 2 hours to about 24 hours.

Similarly, Formula II compounds described in the above paragraph wherein R₄ contains an alkylsulfinyl group may be prepared by oxidation of the corresponding alkylsulfanyl substituted Formula II compound. Typically the
 25 appropriate Formula II compound is treated with one equivalent of a peroxy acid such as meta-chloroperbenzoic acid in an anhydrous halogenated solvent such as dichloromethane at ambient temperature for 1 hour to about 6 hours. The corresponding alkylsulfonyl Formula II compounds can be prepared in an analogous manner using excess peroxy acid.

30 The desired Formula II compounds wherein Steroid is the steroid moiety described above, and C^{1'} and C^{1''} are as defined above and at least one of R₁, R₂, and R₃ is Z-R₄ and R₄ is alkylaminocarbonylalkyl can be prepared from the corresponding carboxy alkyl Formula II compounds through an amidation

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reaction. Typically the amide is formed by reacting the carboxylic acid with a carboxyl activating agent such as a substituted carbodiimide and hydroxybenzotriazole and a primary or secondary amine chosen to give the desired amide product. The reaction is typically performed in an anhydrous aprotic solvent such as dichloromethane at ambient temperature for about 0.5 hours to about 6 hours. The carboxy alkyl Formula II compounds used in this procedure are typically prepared from the corresponding benzyl ester (the preparation of the benzyl ester being described herein) by a hydrogenolysis reaction. Thus the ester is treated with a hydrogenation catalyst such as palladium on carbon in an alcoholic solvent such as methanol and placed under 1 to 4 atmospheres of hydrogen, typically 2 atmospheres, for about 0.5 to about 8 hours.

The desired Formula I compounds wherein Steroid is the steroidal moiety described above, C^{1'} and C^{1''} are as described above and at least one of R₁, R₂ and R₃ is -O-C(=O)-R₄ or -O-C(=O)-N(R⁵)-R₄ may be prepared by acylating the appropriate Formula VI compound wherein Q¹, Q², C^{1'} and C^{1''} are as defined above (See Scheme II). Alternatively, the desired formula II compounds wherein at least one of R₁, R₂ and R₃ is -O-C(=O)-R₄, -O-C(=O)-N(R⁵)-R₄ or -O-C(=S)-N(R⁵)-R₄ may be prepared by acylating the appropriately protected Formula V compound wherein Steroid is the steroidal moiety described above, C^{1'} and C^{1''} are as defined above and C^{6'}, C^{6''} and C^{4''} are each independently substituted with hydroxy or a conventionally protected hydroxyl group such as -OAc (See Scheme I).

A nonselective mixture of esters and carbamoyloxy substitution at R₁ and R₂ is achieved by treating the appropriately protected perhydroxy sugar Formula VI compound with the appropriate acid chloride or isocyanate and an amine base that also acts as an anhydrous solvent such as pyridine in the presence of a drying agent such as molecular sieves at a temperature of about -60°C to about 25°C for about 5 minutes to about 24 hours while the reaction is allowed to warm to ambient temperature. Different products and product mixes result from the variation of the amount of acid chloride or isocyanate used, the length of reaction time and the reactivity of the acid chloride or isocyanate.

Alternatively, a more selective acylation is performed by treating the appropriately protected (e.g., OAc) steroidal glycoside Formula V compound with the appropriate isocyanate or acid chloride in the presence of a base, preferably an

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amin bas such as triethylamin or pyridine and a catalytic amount of an acylation catalyst such as dimethylaminopyridine in an anhydrous, aprotic solvent such as dichloromethane at a temperature of about -20 °C to about 20 °C. The reaction mixture is allowed to warm to ambient temperature for about 10 minutes to about 5 two hours. The carbamoylation can also be achieved by treating the appropriately protected Formula V compound with the appropriate isocyanate in the presence of cuprous chloride in a polar aprotic solvent such as dimethylformamide at ambient temperature for two hours to about 10 hours.

The carbamoylation may also be achieved by treating the appropriately 10 protected Formula V compound with the appropriate isocyanate in the presence of an organotin catalyst such as dibutyl tin dilaurate in an anhydrous aprotic solvent such as dichloromethane at ambient temperature for about 2 hours to about 24 hours.

In addition, the desired Formula II compounds wherein at least one of R_1 , R_2 and R_3 is a carbamoyloxy or thiocarbamoyloxy moiety may be prepared by 15 treatment of the appropriately protected (e.g., OAc) steroidal glycoside Formula V compound with a phosgene equivalent such as carbonyl diimidazole or a thiophosgene equivalent such as thiocarbonyl diimidazole in the presence of a base, preferably an amine base such as diisopropylethylamine in an aprotic, anhydrous solvent such as dichloroethane at a temperature of about 15 °C to about 30°C 20 (typically ambient temperature) for about one to about four hours. The appropriate amine is added and the reaction mixture is stirred at the same temperature for about one hour to about six hours, and heated if necessary to about 40°C to about 60°C for about one to about four hours.

The desired Formula II compounds wherein Steroid is the steroidal moiety 25 described above, $C^{1'}$ and $C^{1''}$ are as described above and at least one of R_1 , R_2 and R_3 is $-NH-C(=O)-R_4$ or $-NH-C(=O)-N(R^5)-R_4$ may be prepared by acylating the appropriately protected Formula III compound wherein Steroid is the steroidal moiety described above, $C^{1'}$ and $C^{1''}$ are as defined above and at least one of R_1 , R_2 , and R_3 is amino or hydroxy.

30 Typically the amide may be prepared by the treatment of the appropriately protected (e.g., OAc) steroidal glycosidyl amine with the appropriate acid anhydride or acid chloride in the presence of a base, preferable an amine base such as

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triethylamin in an anhydrous, aprotic solvent such as dichloromethane for about one to about three hours at a temperature of about 0°C to about 25°C.

Alternatively, the ureas may be prepared by the treatment of the appropriately protected (e.g., OAc) steroidal glycosidyl amine with the appropriate isocyanate in
5 an anhydrous, aprotic solvent such as dichloromethane for about one to about three hours at a temperature of about 0°C to about 25°C.

The desired Formula III compound (which happens in this case to be a Formula II compound) wherein Steroid is the steroidal moiety described above, C^{1'} and C^{1''} are as defined above and at least one of R₁, R₂, and R₃ is amino or azido
10 may be prepared from the corresponding Formula IV mesylated or halogenated compounds by azide displacement followed if necessary by reduction.

Typically the mesylate or halogen compound is exposed to a metal azide such as sodium azide in a polar, aprotic solvent such as N, N-dimethylformamide (in an inert atmosphere) at a temperature of about 70 °C to about 150 °C for about two to
15 about 10 hours. The preparation of such mesylate compounds are described above for the lithium halide halogenation. Typically the azido compounds are reduced to the corresponding amines by exposure to hydrogen gas in the presence of a noble metal catalyst such as palladium on carbon at ambient temperature for about four to about forty eight hours, under pressures of about one to about three atmospheres.

20 The desired Formula V compound (appropriately protected to yield the desired substitution described above) wherein Steroid is the steroidal moiety described above, C^{1'} and C^{1''} are as defined above and C^{6'}, C^{6''} and C^{4''} are each independently substituted with hydroxy or a conventionally protected hydroxyl group such as -OAc may be prepared by conventional protecting group methods of
25 organic synthesis known to those skilled in the art from the corresponding Formula VI compounds wherein Q¹, Q², C³, C⁵, C²⁵, C^{1'} and C^{1''} are as defined above. For a general description of protecting groups and their use, see T. W. Greene, Protective Groups in Organic Synthesis, John Wiley & Sons, New York, 1991. In addition, as an aid to the preparation of the above protected steroidal glycosides, the following
30 paragraphs describe the preparation of various protected steroidal glycosides from their hydroxy analogues using a combination of differentially selective protecting groups and sequential protection reactions.

For example, the desired Formula V compound wherein Steroid is the steroidal moiety described above, C^{1'} and C^{1''} are as defined above, C^{6'} and C^{6''} are substituted with hydroxy and C^{4''} is substituted with OP where P is acyl may be conveniently prepared from the corresponding perhydroxy steroidal glycoside by silylation, acylation and desilylation. The appropriate Formula VI compound is reacted with a base, preferably an amine base such as imidazole, a bulky silylating agent selected to afford the desired selective protection such as a trisubstituted silylhalide, preferably t-butyldiphenylsilyl chloride and a catalytic amount of a silylation catalyst such as dimethylaminopyridine in an anhydrous, aprotic solvent such as N, N-dimethyl-formamide at about -20 °C to about 10 °C followed by ambient temperature stirring for about one to about six hours. Upon completion of the silylation, a base, preferably an amine base such as pyridine and an acylating agent selected to afford the desired acyl protecting group defined above such as acetic anhydride are added at ambient temperature and pressure for about three to about twelve hours to achieve acetylation to prepare the desired protected compound (e.g., Formula IIB compound). The resulting product is treated with hydrogen fluoride in an anhydrous, aprotic solvent such as pyridine at about -20 °C to about 10 °C followed by ambient temperature stirring for about two to about six hours to prepare the desired selectively protected compound (e.g., Formula IIA compound). This product contains hydroxyl groups at the C^{6'} and C^{6''} positions which can be further differentiated by reaction with one equivalent of a protecting group such as acetic anhydride in the presence of a base, such as pyridine at ambient temperatures for about 1 to about 4 hours. This procedure gives a mixture of Formula V compounds which contain a single hydroxyl group at either the C^{6'} or the C^{6''} position which can be separated chromatographically.

In addition, the desired Formula V compound wherein Steroid is the steroidal moiety described above, C^{1'} and C^{1''} are as defined above, C^{6'} and C^{6''} are substituted with hydroxy and C^{6'} is substituted with OP where P is acyl may be conveniently prepared from the corresponding perhydroxy steroidal glycoside by ketalization, acylation and deketalization. The appropriate Formula VI compound is reacted with an acetal or ketal selected to afford the desired cyclic protecting group defined above such as benzaldehyde dimethyl acetal or anisaldehyde dimethyl acetal in the presence of a catalytic amount of a strong acid such as

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camphorsulfonic acid in an anhydrous, aprotic solvent such as chloroform or dichloroethane under reflux conditions for about two to about six hours at ambient pressure. Upon completion of the ketalization, a base preferably an amine base such as pyridine, a catalytic amount of an acylation catalyst such as

- 5 dimethylaminopyridine and an acylating agent selected to afford the desired acyl protecting group defined above such as acetic anhydride or chloroacetic anhydride were added at a temperature of about -20 °C to about 10 °C followed by ambient temperature stirring for about one to about twelve hours to prepare the desired protected compound. The resulting product is treated with 80% acetic acid in water
- 10 at about 50 °C to about reflux conditions for about one to about four hours or with trifluoroacetic acid in a mixture of dichloromethane and methanol at ambient temperature for about two to about eight hours to prepare the desired protected compound (e.g., Formula IIA compound).

- This product can further be converted to the Formula V compound wherein
- 15 C^{6'} and C^{6''} are substituted with OP where P is an acyl or silyl protecting group and C^{4'} is substituted with OH by a selective silylation reaction. Typically the silylation is performed by treating the appropriate Formula V compound wherein C^{4'} and C^{6'} are substituted with OH and C^{6'} is substituted with OP where P is an acyl protecting group with a silylating agent such as tert-butyldimethylsilyl chloride and a base
 - 20 preferably an amine base such as imidazole in a polar aprotic solvent such as dimethyl formamide at ambient temperature for about 12 hours to about 48 hours.

- The desired Formula V compound wherein Steroid is the steroidal moiety described above, C^{1'} and C^{1''} are as defined above, C^{6'} and C^{4'} are substituted with hydroxy and C^{6'} is substituted with OP where P is an ether protecting group may be
- 25 conveniently prepared from the corresponding perhydroxy steroidal glycoside by ketalization, etherification and deketalization. The ketalization is performed as described above. Upon completion, the solvent is removed and replaced with a polar aprotic solvent such as dimethyl-formamide. The appropriate alkyl halide is added such as benzyl bromide, followed by a strong base such as sodium hydride
 - 30 at a temperature of about -20°C to about 0°C for about 1 hour to about 12 hours. The deketalization is performed as described above.

The desired Formula VI compounds wherein Q¹, Q², C³, C⁵, C²⁵, C^{1'} and C^{1''} are as defined above may be prepared from the corresponding Formula VII

Formula VIII peracetylated steroidal glycoside by the deacetylation process described above. For those Formula VI compounds wherein the C^{1'} anomeric oxygen is alpha an anomerization is performed on the corresponding Formula VII compound wherein the C^{1'} anomeric oxygen is beta prior to deacetylation. The stereochemical
5 terms alpha and beta refer to the configuration of the attachment carbon of the sugar. Typically the anomerization is performed by treatment with a mineral acid such as hydrobromic acid in an anhydrous aprotic solvent such as methylene chloride at temperatures of 20 °C to about 40 °C (typically ambient) for at least 24 hours, typically to several days.

10 The desired Formula VII compounds wherein Q¹, Q², C³, C⁵ and C²⁵ are as described above may be prepared by coupling the appropriate acetylated sugar halide (e.g., bromide) and steroid. More specifically, for those Formula VII compounds where the sugar is other than beta-D-maltosyl, a zinc fluoride promoted coupling of the appropriate Formula IX compound (wherein Q¹, Q², C³, C⁵ and C²⁵
15 are as described above) and peracetylated sugar halide is used. For those Formula VII compounds where the sugar is beta-D-maltosyl, a mercuric bromide and mercuric cyanide promoted coupling of the appropriate Formula X compound (e.g., trimethyl silyl ether of the Formula IX compound wherein Q¹, Q², C³, C⁵ and C²⁵ are as described above) and peracetylated sugar halide is used.

20 Generally, the zinc fluoride promoted coupling of the Formula IX compound and the peracetylated sugar bromide occurs in a non-protic, anhydrous reaction-inert solvent (e.g., acetonitrile) at a temperature of about 20 °C to about 100 °C for about 0.5 to about 12 hours. Typically about 0.5 to about 4 equivalents (based on Formula IX compound) zinc fluoride is used and about 0.5 to about 3 equivalents
25 acetylated sugar bromide is used. Preferably the coupling is acid catalyzed and it is especially preferred that hydrohalic acid generated during the reaction is used as the acid catalyst. The desired compounds may be prepared at pressures of 0.5 to 50 psi, although typically ambient pressures are used. In a preferred isolation technique the glycosides may be precipitated from the crude filtered reaction mixture
30 (e.g., acetonitrile product solution) by the addition of about 25% to 75% water and the remainder alcohol (e.g., methanol). Precipitation of the product from aqueous methanol/acetonitrile requires less processing than an extractive isolation, and provides a product of great purity. Generally, the mercuric bromide and mercuric

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cyanid promoted coupling of the Formula X compound and the acetylated beta-D-maltosyl bromide is performed in an aprotic, anhydrous solvent such as methylene chloride at a temperature of about 20 °C to about 100 °C for about 0.5 to about 6 hours. Typically about 0.5 to about 4 equivalents (based on acetylated beta-D-

5 maltosyl bromide) mercuric bromide and mercuric cyanide is used and about 0.5 to about 3 equivalents peracetylated beta-D-maltosyl bromide is used. The desired compounds may be prepared at pressures of 0.5 to 50 psi, although typically ambient pressures are used. Preferably they are isolated as described for the zinc fluoride promoted coupling of the Formula IX compound.

- 10 The desired Formula X compounds wherein Q¹, Q², C³, C⁵ and C²⁵ are as described above may be prepared by silylating the appropriate Formula IX compound wherein Q¹, Q², C³, C⁵ and C²⁵ are as described above. Generally the Formula IX compound, a base such as triethylamine and an activated trialkylsilyl compound (e.g., trimethylsilyl trifluoromethane sulfonate of trimethylsilyl chloride) are
- 15 reacted in an aprotic, anhydrous solvent such as methylene chloride at a temperature less than about 10 °C for about 0.5 hour to about two hours.

In general, the procedures described above may be combined thus providing Formula I compounds wherein the R₁, R₂ and/or R₃ groups are dissimilar (e.g., halogenation followed by carbamoylation).

- 20 The starting materials for the above described reaction schemes (e.g., alkoxyalkyl halide, acid anhydride, peracetylated sugar halides, acid chlorides, isocyanates, steroids, amines, trialkylsilylchlorides, carbonyl diimidazoles, thiocarbonyl diimidazoles, acid derivatives, acetals, ketals, protecting groups) are readily available or can be easily synthesized by those skilled in the art using
- 25 conventional methods of organic synthesis. For example some of the compounds of this invention require the synthesis of substituted amines and carboxylic acids which eventually will become R₄ groups. Such preparations are standard and known to those skilled in the art.

- In addition, as an aid to the preparation of the above steroids, the following
- 30 paragraphs describe the preparation of the various Formula IX compounds from 12-keto analogs (e.g., hecogenin). Literature references for the preparation of Formula IX steroid compounds (wherein Q¹ is methylene and Q² is carbonyl and the

stereochemistry of the C⁵ hydrogen (or lack of the C⁵ hydrogen) and C²⁵ carbon are as defined below) are described in Table I.

TABLE I

Formula IX Compounds Where Q¹ is Methylene, Q² is carbonyl and the C³ Hydroxy Group is Beta

5

C ⁵ hydrogen	C ²⁵	C ⁵ -C ⁶ double bond	Reference
α	R	No	Marker et. al., J. Am. Chem. Soc. (1947) 69, 2167.
α	S	No	Callow & James J. Chem. Soc. (1955) 1671.
10 β	R	No	Marker et. al., J. Am. Chem. Soc. (1947) 69, 2167.
β	S	No	Kenney & Wall J. Org. Chem. (1957) 22, 468.
—	R	Yes	Walens, et al., J. Org. Chem. (1957) 22, 182.
—	S	Yes	Walens, et al., J. Org. Chem. (1957) 22, 182.

15 The following paragraphs describe and/or give literature references for the preparation of the various steroids used as starting materials (i.e., the alternative stereochemistry at the C³ position and the oxygenation and different epimers at C¹¹ (Q¹) and C¹² (Q²) from the above Formula IX compounds described in Table I. In general the preparation of the different oxygenated steroids is independent of the stereochemistry at the C³, C⁵ (or lack of the C⁵ hydrogen) and C²⁵ positions. Thus, once the appropriate stereochemistry at the C³, C⁵ (or lack of the C⁵ hydrogen) and C²⁵ positions are achieved where Q¹ is methylene and Q² is carbonyl, the various oxygenated compounds at Q¹ and Q² may be prepared therefrom.

25 Some of the preparation methods described herein will require protection of remote functionality (i.e., C¹¹ (Q¹) and C¹² (Q²)). The need for these protecting groups will vary depending on the nature of the remote functionality and the conditions of the preparation methods. This need is readily determined by one skilled in the art. For a general description of protecting groups and their use, see T.W. Greene, Protective Groups in Organic Synthesis, John Wiley & Sons, New York, 1991.

30

The Formula IX compounds where in Q¹ is methylene, Q² is carbonyl and the C³ hydroxy is beta may be converted to the corresponding Formula IX compounds where the C³ hydroxy is alpha by the following two procedures. These preparative methods may be used independent of the C²⁵ stereochemistry.

- 5 The carbonyl is protected as a ketal (e.g., ethylene ketal), by reacting the steroid with ethylene glycol and an acid catalyst according to the procedure of Engel and Rakhit, Can. J. Chem, 40, 2153, 1962. When the C⁵ hydrogen is alpha, the C³ hydroxy group is oxidized to the ketone using pyridinium chloro chromate (PCC) in methylene chloride at ambient conditions. Then the C³ ketone is reduced
10 with a sterically hindered reducing agent such as K-Selectride® reducing agent, at low temperature in tetrahydrofuran to give the C³ alpha alcohol according to Gondos and Orr, J. Chem. Soc. Chem. Commun. 21, 1239, 1982. The Q² (C¹²) protecting group is removed with acid, such as hydrochloric acid, in an appropriate solvent such as acetone.

- 15 For those compounds wherein the C⁵ hydrogen is beta the same procedures are used as were used when the C⁵ hydrogen is alpha except the C³ ketone is reduced using sodium borohydride in ethanol to furnish the C³ alpha alcohol.

- Reaction Scheme III illustrates the reaction pathways to achieve the Formula IX compounds wherein Q¹ (C¹¹) and Q² (C¹²) are defined above starting from the
20 Formula IX compound wherein Q¹ is methylene and Q² is carbonyl.

In general, preparation methods for these compounds may be found in L.F. Fieser and M. Fieser, Steroids, Reinhold Pub. Corp., New York, 1959 and references therein, however, the following descriptive text (which is keyed to Reaction Scheme III) provides specific guidance.

- 25 Briefly according to Reaction Scheme III method 1, the starting material is acetylated and brominated according to the procedure described in J. Chem. Soc., 1956, 4344. This intermediate is then reduced with lithium aluminum hydride and treated with silver oxide by a procedure similar to that described in Helv. Chim. Act., 1953, 36, 1241. The resulting β -11,12-epoxide is opened with trichloroacetic acid,
30 saponified and reduced with zinc and acetic acid using the procedure described in J. Chem. Soc., 1956, 4330 to give the product shown for method 1.

In method 2, the starting material is selectively acetylated using the procedure described in J. Chem. Soc., 1956, 430. Using the procedure described

in Org. Syn., 1976, 55, 84, the resulting product is oxidized with chromium trioxide and pyridine. Using the procedure described in Synthesis, 1973, 790, the resulting product is saponified with potassium cyanide in water, methanol and THF to give the product shown for method 2. In method 3, the starting material is converted to the
5 corresponding toluenesulfonylhydrazone which is in turn treated with sodium methoxide using a procedure similar to that described in J. Am. Chem. Soc., 1954, 76, 4013. The resulting 11-ene product is oxidized with osmium tetroxide and N-methylmorpholine-N-oxide according to the procedure described in Tetrahedron Letters, 1976, 1973 to give the product shown for method 3.

10 In method 4, the starting material is monobrominated using a procedure described in US Pat. No. 3,178,418. Hydrolysis of this intermediate using the procedure described in J. Chem. Soc., 1956, 4330 gives the product shown for method 4.

In method 5, the starting material is reduced with lithium aluminum hydride
15 according to the procedure described in J. Am. Chem. Soc., 1951, 73, 1777 to give the product shown.

In method 6, the starting material is reduced with lithium and ammonia according to the procedure described in J. Am. Chem. Soc., 1953, 75, 1282 to give the product shown.

20 In method 7, the starting material is acetylated according to the procedure described in J. Am. Chem. Soc., 1955, 77, 1632 to give a mixture of acetates from which the 3,11-diacetate can be isolated. The unprotected 12-alcohol is then oxidized with chromium trioxide and pyridine according to the procedure described in Org. Syn., 1976, 55, 84. Saponification of the acetates gives the product shown
25 for method 7.

In method 8, the starting material is diacetylated using the procedure described in J. Chem. Soc., 1956, 4330. The diacetate is reduced with calcium and ammonia using the procedure described in J. Chem. Soc., 1956, 4334 to give the product shown for method 8.

30 In method 9, the starting material is reduced with lithium and ammonia according to the procedure described in J. Am. Chem. Soc., 1953, 75, 1282 to give the product shown.

In method 10, the starting material is reduced with lithium aluminum hydride according to the procedure described in J. Am. Chem. Soc., 1951, 73, 1777 to give the product shown. In method 11, the starting material is selectively protected at the 3-alcohol with t-butyldimethylchlorosilane and imidazole using the procedure described in J. Am. Chem. Soc., 1972, 94, 6190. Using the procedure described in Org. Syn., 1976, 55, 84, the product is oxidized with chromium trioxide and pyridine. The 3-alcohol is then desilylated with hydrofluoric acid in acetonitrile using the procedure described in J. Am. Chem. Soc., 1972, 94, 6190 to give the product shown for method 11.

10 In method 12, the starting material is selectively protected at the 3-alcohol with t-butyldimethylchlorosilane and imidazole using the procedure described in J. Am. Chem. Soc., 1972, 94, 6190. The resulting intermediate is reduced with lithium aluminum hydride using the procedure described in J. Am. Chem. Soc., 1951, 73, 1777. The resulting intermediate is selectively acetylated on the 12-alcohol, silylated
15 on the 11-alcohol with trimethylsilyltriflate and 2,6-lutidine using the procedure described in Tetrahedron Letters, 1981, 22, 3455, and then deacetylated at the 12-alcohol with lithium aluminum hydride and an aqueous ammonium chloride quench. The 12-alcohol is oxidized with chromium trioxide and pyridine in methylene chloride using the procedure described in Org. Syn., 1976, 55, 84, and then desilylated with
20 hydrofluoric acid in acetonitrile using the procedure described in J. Am. Chem. Soc., 1972, 94, 6190 to give the product shown in method 12.

The compounds of Formula I which have been obtained and have asymmetric carbon atoms (e.g., some of the components of the carbamoyl moieties such as substituted amino groups) can be separated into their diastereomers and
25 enantiomers on the basis of their physical chemical differences or optical qualities by methods known per se, for example, by chromatography and/or fractional crystallization. All such isomers, including diastereomers and enantiomers are considered as part of this invention.

The compounds of this invention where R₄ contains an amine group are
30 basic and they form acid salts. All such acid salts are within the scope of this invention and they can be prepared by conventional methods. For example, they can be prepared simply by contacting the acidic and basic entities, usually in a stoichiometric ratio, in either an aqueous, non-aqueous or partially aqueous

m dium, as appropriate. The salts are recovered either by filtration, by precipitation with a non-solvent followed by filtration, by vaporation of the solvent, or, in the case of aqueous solutions, by lyophilization, as appropriate.

In addition, many of the compounds of this invention may be isolated as
5 hydrates.

The compounds of this invention are potent inhibitors of cholesterol absorption and thus are all adapted to therapeutic use as hypercholesterolemia controlling agents in mammals, particularly humans. Since hypercholesterolemia is closely related to the development of generalized cardiovascular, cerebral vascular
10 or peripheral vascular disorders, secondarily these compounds prevent the development of atherosclerosis particularly arteriosclerosis.

The hypercholesterolemia controlling activity of these compounds may be demonstrated by methods based on standard procedures. For example, the *in vivo* activity of these compounds in inhibiting intestinal absorption of cholesterol may be
15 determined by the procedure of Melchoir and Harwell (J. Lipid Res., 1985, 26, 306-315).

Activity can be determined by the amount of hypocholesterolemic agent that reduces the cholesterol absorption, relative to the control, in male golden Syrian hamsters. Male golden Syrian hamsters are administered either a cholesterol-free
20 diet (control animals) or a diet supplemented with 1% cholesterol and 0.5% cholic acid for 4 days. The following day the animals are fasted for 18 hours, then administered a 1.5 ml oral bolus of water containing 0.25% methylcellulose, 0.6% Tween™ 80 and 10% ethanol (control animals) or an oral bolus that contains, in addition, the desired concentration of the compound to be tested. Immediately
25 following bolus administration, the animals receive a second 1.5 ml oral bolus of liquid hamster diet containing 1% [³H] cholesterol (2.0 μ Ci/animal; 210 dpm/nmol) and 0.5% cholic acid, and are fasted for an additional 24 hours. At the end of this second fasting period animals are sacrificed, livers are excised, saponified and aliquots are decolorized by addition of hydrogen peroxide, and assessed for
30 radioactivity. Total hepatic radioactivity is calculated based on measured liver weights. The degree of cholesterol absorption is expressed as a percentage of the total radioactivity administered as an oral bolus that is present in the liver 24 hours following bolus administration.

Anti-atherosclerosis effects of the compounds can be determined by the amount of agent that reduces the lipid deposition in the rabbit aorta. Male New Zealand White rabbits are fed a diet containing 0.4% cholesterol and 5% peanut oil for 4 days (meal-fed once per day). Rabbits are bled from the marginal ear vein and total plasma cholesterol values are determined from these samples. The rabbits are then assigned to treatment groups so that each group has a similar mean \pm s.d. for total plasma cholesterol concentration. After group assignment, rabbits are dosed daily with compound given as a dietary admix or on a small piece of gelatin based confection. Control rabbits receive only the dosing vehicle be it the food or the gelatin confection. The cholesterol/peanut oil diet is continued along with the compound administration throughout the study. Plasma cholesterol values can be determined at any point during the study by obtaining blood from the marginal ear vein. After 5 months, the rabbits are sacrificed and the aortae are removed from the thoracic arch to the branch of the iliac arteries. The aortae are cleaned of adventitia, opened longitudinally and then stained with Sudan IV as described by Holman et al. (Lab. Invest. 1958, 7, 42-47). The percent of the surface area stained is quantitated by densitometry using an Optimas Image Analyzing System (Image Processing Systems). Reduced lipid deposition is indicated by a reduction in the percent surface area stained in the drug group in comparison with the control rabbits.

Administration of the compounds of this invention can be via any method which delivers the compounds to the intestinal lumen. These methods include oral routes, intraduodenal routes etc.

The amount of steroidal glycoside administered will, of course, be dependent on the subject being treated, on the severity of the affliction, on the manner of administration and on the judgement of the prescribing physician. However, an effective dosage is in the range of 0.005 to 20 mg/kg/day, preferably 0.01 to 5 mg/kg/day, most preferably 0.01 to 1 mg/kg/day. For an average 70 kg human, this would amount to 0.00035 to 1.4 g/day, preferably 0.0007 to 0.35 g/day, most preferably 0.0007 to 0.07 g/day. In one mode of administration the compounds of this invention are taken with meals.

For oral administration, which is preferred, a pharmaceutical composition can take the form of solutions, suspensions, tablets, pills, capsules, powders, sustained release formulations and the like.

Depending on the intended mode of administration, the pharmaceutical compositions may be in the form of solid, semi-solid or liquid dosage forms, such as, for example, tablets, pills, capsules, powders, liquids, suspensions, or the like, preferably in unit dosage forms suitable for single administration of precise dosages.

- 5 The pharmaceutical compositions will include a conventional pharmaceutical carrier or excipient and a compound according to the invention as an active ingredient. In addition, it may include other medicinal or pharmaceutical agents, carriers, adjuvants, etc.

- Pharmaceutical compositions according to the invention may contain 0.1%-
10 95% of the compound, preferably 1%-70%. In any event, the composition or formulation to be administered will contain a quantity of a compound according to the invention in an amount effective to alleviate the signs of the subject being treated, i.e., hypercholesterolemia or atherosclerosis.

- For solid pharmaceutical compositions, conventional non-toxic solid carriers
15 include, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium carbonate, and the like.

- Liquid pharmaceutically administrable compositions can be prepared by dissolving or dispersing, or otherwise preparing a compound according to this
20 invention and mixing it optionally with a pharmaceutical adjuvant in a carrier, such as, for example, water, saline, aqueous dextrose, glycerol, ethanol, and the like, to thereby form a solution or suspension.

- Methods of preparing various pharmaceutical compositions with a certain amount of active ingredient are known, or will be apparent, to those skilled in this
25 art. For examples, see Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pa., 15th Edition (1975).

- It should be understood that the invention is not limited to the particular embodiments shown and described herein, but that various changes and modifications may be made without departing from the spirit and scope of this novel
30 concept as defined by the following claims.

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Exempl 1(3 β ,5 α , 25R)-3-[(4'-[2-fluoro-phenylcarbamoyl]-
- β -D-cellobiosyl)oxy]-spirostan-11-oneDESILYLATION

- 5 Tetra-n-butyl ammonium fluoride (0.92 mL of a 1M solution in THF, 0.92 mmol) was added to a solution (3 β ,5 α , 25R)-3-[(4'-[2-fluoro-phenylcarbamoyl]-6'-triisopropylsilyl- β -D-cellobiosyl)oxy]-spirostan-11-one (321 mg, 0.31 mmol) in THF (5mL) at room temperature. After 30 min, the mixture was concentrated in vacuo and the residue was dissolved in methanol (1 mL) and the product was precipitated
- 10 by the addition of water (5mL). The solid was collected by vacuum filtration, washed with water and dried to afford 190 mg of the title product as a colorless solid (70%). m.p. >265°C. FAB MS: 914 (M+Na)⁺. Analysis calc. for C₄₆H₆₆FNO₁₅ + 0.4 H₂O: C 61.44; H 7.49; N 1.56. Found: C 61.14; H 7.45; N 1.59.

Example 215 (3 β ,5 α ,25R)-3-[(6',6"-Bis-[phenylcarbamoyl]- β -D-cellobiosyl)oxy]-spirostan-11-oneDEACETYLATION USING SODIUM METHYLATE

- To a solution of (3 β ,5 α ,25R)-3-[(6',6"-bis-[phenylcarbamoyl]-penta acetyl- β -D-cellobiosyl)oxy]-spirostan-11-one (0.67 g, 0.56 mmol) in tetrahydrofuran (4 mL) and methanol (4 mL), sodium methylate (37 mg) was added. The reaction was stirred for
- 20 45 minutes at room temperature under nitrogen atmosphere. Upon completion, the reaction was quenched with acetic acid (2-3 drops) and concentrated in vacuo. The crude material was purified via flash chromatography (95% chloroform: 5% methanol). The isolated product was concentrated in vacuo to partial dryness and water was added causing a precipitate to form. The precipitated product was filtered,
- 25 washed with water and oven-dried to afford 189 mg (33.9 % yield) of the title compound. ¹HNMR (250 MHz, DMSO-d₆) δ 9.65 (2, 1H); 8.35 (s, 1H); 7.6-7.9 (m, 10 H); 5.4-5.1 (m, 4H); 4.65-2.95 (m, 19H); 2.6-1.0 (m, 25H); 0.9 (d, 3H, J=8 Hz); 0.87 (s, 3H); 0.75 (d, 3H, J=8 Hz); 0.6 (2, 3H). FAB MS: 1015 (M+Na)⁺; Analysis: calculated for C₆₃H₇₂N₂O₁₆·1 H₂O
- 30 C 61.85, H 7.44, N 2.72; found C 62.23, H 7.29, N 2.59; m.p. 244-246°C (dec).

Examples 3-77

The following compounds were prepared from the appropriate starting material in an analogous manner using the above procedure.

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Example)	Name			
m.p.	M.S.	formula	elemental analysis	
=====				
3)	<u>(3β,5α,25R)-3-[(6',6"-dideoxy-6',6"-difluoro-β-D-cellobiosyl)oxy]-spirostan-11-one</u>			
5	>270°C	759(M+H) ⁺	C ₃₉ H ₆₀ F ₂ O ₁₂ +1.0 H ₂ O	calc. C 60.29; H 8.04 found C 60.12; H 8.11
4)	<u>(3β,5α,25R)-3-[(6"-deoxy-6"-fluoro-β-D-cellobiosyl)oxy]-spirostan-11-one</u>			
10	268-269°C	757(M+H) ⁺	C ₃₉ H ₆₁ FO ₁₃	calc C 61.89; H 8.12 found C 61.68; H 7.77
5)	<u>(3β,5α,25R)-3-[(6',6"-dideoxy-6',6"-difluoro-β-D-maltosyl)oxy]-spirostan-11-one</u>			
15	>250°C	759(M+H) ⁺	C ₃₉ H ₆₀ F ₂ O ₁₂ +0.5 H ₂ O	calc. C 61.00; H 8.01 found C 60.81; H 7.93
6)	<u>(3β,5α,25R)-3-[(6',6"-ethoxymethyl-β-D-cellobiosyl)oxy]-spirostan-11-one</u>			
	216-218°C	893(M+Na) ⁺	C ₄₅ H ₇₄ O ₁₆	calc. C 61.42; H 8.59
	(dec)		+0.5 H ₂ O	found C 61.58; H 8.76
7)	<u>(3β,5α,25R)-3-[(6',6"-dideoxy-6',6"-dichloro-β-D-cellobiosyl)oxy]-spirostan-11-one</u>			
20	260°C	791(M+H) ⁺	C ₃₉ H ₆₀ Cl ₂ O ₁₂	calc. C 58.50; H 7.68
	(dec)		+0.5 H ₂ O	found C 58.59; H 7.69
8)	<u>(3β,5α,25R)-3-[(6',6"-dideoxy-6',6"-diiodo-β-D-cellobiosyl)oxy]-spirostan-11-one</u>			
25	200°C	975(M+H) ⁺	C ₃₉ H ₆₀ I ₂ O ₁₂	calc. C 47.62; H 6.25
	(dec)		+0.5 H ₂ O	found C 47.73; H 5.99
9)	<u>(3β,5α,25R)-3-[(6',6"-Bis[2,4-difluoro-phenylcarbamoyl]-β-D-cellobiosyl)oxy]-spirostan-11-one</u>			
30	250°C	1087(M+Na) ⁺	C ₅₃ H ₈₈ F ₄ N ₂ O ₁₈ +0.6H ₂ O	calc. C 59.17; H 6.48; N 2.60 found C 58.92; H 6.61; N 2.59

10) (3 β ,5 α ,25R)-3-[(6',6"-dideoxy- β -D-cellobiosyl)oxy]-spirostan-11-on

>275°C	724(M+H)⁺	C₃₉H₈₂O₁₂	calc.	C 63.60; H 8.69
		+0.75 H₂O	found	C 63.61; H 8.94

5 11) (3 β ,5 α ,25R)-3-[[6',6"-Bis(pivaloyl)- β -D-cellobiosyl]oxy]-spirostan-11-one

224-226°C	923(M+H) ⁺	HRMS calc. for C ₄₉ H ₇₉ O ₁₈	923.5368
(dec)		found	923.5284

12) (3 β ,5 α ,25R)-3-[6'-[2,4-difluoro-phenylcarbamoyl]- β -D-cellobiosyl)oxy]-spirostan-

10 11-one

>265°C	910(M+H)⁺	C₄₆H₆₅F₂NO₁₅	calc.	C 60.71; H 7.20; N 1.54
			found	C 60.36; H 6.89; N 1.28

13) (36,5a,25R)-3-[(4',6'-Bis[2-methoxy-phenylcarbamoyl]- β -D-cellobiosyl)oxy]-

15 spirostan-11-one

>265°C	1075(M+Na)⁺ C₅₅H₇₆N₂O₁₈	calc.	C 60.15; H 7.43; N 2.55
	+ 2.5 H₂O	found	C 60.23; H 7.14; N 2.36

14) (3 β ,5 α ,25R)-3-[(6"-[2-methoxy-phenylcarbamoyl]- β -D-cellobiosyl)oxy]-spirostan-

20 11-one

247-248°C	926(M+Na) ⁺	C ₄₇ H ₆₇ NO ₁₈	calc.	C 61.22; H 7.76; N 1.52
		+ 1 H ₂ O	found	C 61.33; H 7.56; N 1.32

15) (3R,5R)-3-[(4',6'-Bis[4-hydroxy-butylcarbamoyl]-β-D-cellobiosyl)oxy]-

25 spirostan-11-one

228-230°C	1007(M+Na) ⁺ C ₄₇ H ₈₀ N ₂ O ₁₈	calc.	C 57.13; H 8.31; N 2.72
	+ 2.5 H ₂ O	found	C 56.98; H 8.42; N 2.34

16) (38,5g,25R)-3-[(4",6"-Bis[2,6-dimethyl-phenylcarbamoyl]-β-D-cellobiosyl)oxy]-

30 spirostan-11-one

>250°C	1071((M+Na) ⁺ C ₅₇ H ₈₀ N ₂ O ₁₆	calc.	C 63.12; H 7.75; N 2.58
	+2 H ₂ O	found	C 62.98; H 7.88; N 2.60

- 17) (3 β ,5 α ,25R)-3-[(4',6'-Bis[2,5-difluoro-phenylcarbamoyl]- β -D-cellobiosyl)oxy]-spirostan-11-one
 >250°C 1087(M+Na)⁺ C₅₃H₈₈F₄N₂O₁₆ calc. C 57.84; H 6.54; N 2.54
 +2 H₂O found C 57.54; H 6.73; N 2.52
- 5
- 18) (3 β ,5 α ,25R)-3-[(6',6'-dideoxy-6',6'-dichloro- β -D-lactosyl)oxy]-spirostan-11-one
 243-244°C 813(M+Na)⁺ C₃₉H₆₀Cl₂O₁₂ calc. C 57.21; H 7.75
 +1.5 H₂O found C 57.49; H 7.52
- 10 19) (3 β ,5 α ,12 β ,25R)-3-[(4',6'-Bis[2-fluoro-phenylcarbamoyl]- β -D-cellobiosyl)oxy]12-hydroxy-spirostan-11-one
 211.5-212°C 1067(M+Na)⁺ C₅₃H₇₀F₂O₁₇ calc. C 58.88 H 6.90 N 2.59
 +2H₂O found C 59.09 H 6.81 N 2.86
- 15 20) (3 β ,5 α ,12 β ,25R)-3-[(6',6'-dideoxy-6',6'-difluoro- β -D-cellobiosyl)oxy]12-hydroxy-spirostan-11-one
 239-40°C 797(M+Na)⁺ HRMS calc. for C₃₉H₆₀F₂O₁₃Na: 797.3825
 (dec) found 797.3900
- 20 21) (3 β ,5 α ,12 β ,25R)-3-[(6'-deoxy-6'-fluoro- β -D-cellobiosyl)oxy]12-hydroxy-spirostan-11-one
 268-69°C 794(M+Na)⁺ HRMS calc. for C₃₉H₆₁FO₁₄Na: 795.3943
 (dec) found 795.3881
- 25 22) (3 β ,5 α ,25R)-3-[(4',6'-Bis[2-methoxycarbonyl-phenylcarbamoyl]- β -D-cellobiosyl)oxy]-spirostan-11-one
 245-246°C dec 1131 (M+Na)⁺ C₅₇H₇₆N₂O₂₀ calc. C 60.73; H 6.97; N 2.49
 +1H₂O found C 60.45; H 6.81; N 2.38
- 30 23) (3 β ,5 α ,25R)-3-[(6'-[4-phenoxy-phenylcarbamoyl]- β -D-cellobiosyl)oxy]-spirostan-11-one
 264-265°C 988 (M+Na)⁺ C₅₂H₇₁NO₁₆ calc. C 62.89; H 7.51; N 1.41
 +1.5H₂O found C 62.97; H 7.20; N 1.65

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- 24) (3 β ,5 α , 25R)-3-[(4',6'-Bis[allylcarbamoyl]- β -D-cellobiosyl)oxy]-spirostan-11-one
 255-260°C dec 943 (M+Na) ⁺ C₄₇H₇₂N₂O₁₈ calc. C 59.54; H 7.97; N 2.95
 +1.5H₂O found C 59.74; H 8.28; N 2.91
- 5 25) (3 β ,5 α , 25R)-3-[(4',6'-Bis[3,5-dimethoxy-phenylcarbamoyl]- β -D-cellobiosyl)oxy]-spirostan-11-one
 263-264°C 1135 (M+Na) ⁺ C₅₇H₈₀N₂O₂₀ calc. C 59.76; H 7.35; N 2.45
 +1.8H₂O found C 59.93; H 7.00; N 2.26
- 10 26) (3 β ,5 α , 25R)-3-[(6'-acetamido-6'-deoxy-4'-[2-fluoro-phenylcarbamoyl]- β -D-cellobiosyl)oxy]-spirostan-11-one
 >265°C 955 (M+Na) ⁺ C₄₈H₆₉FN₂O₁₅ calc. C 60.62; H 7.52; N 2.95
 +1H₂O found C 60.62; H 7.30; N 2.89
- 15 27) (3 β ,5 α , 25R)-3-[(6'-acetamido-6'-deoxy- β -D-cellobiosyl)oxy]-spirostan-11-one
 >265°C 818 (M+Na) ⁺ C₄₁H₆₅NO₁₄ calc. C 60.50; H 8.30; N 1.72
 +1H₂O found C 60.36; H 7.96; N 1.67
- 20 28) (3 β ,5 α , 25R)-3-[(4',6'-Bis[phenyl-thiocarbamoyl]- β -D-cellobiosyl)oxy]-spirostan-11-one
 265-268°C dec 1047 (M+Na) ⁺ C₅₃H₇₂N₂O₁₄S₂ calc. C 60.49; H 7.18; N 2.66
 +1.5H₂O found C 60.60; H 7.08; N 2.57
- 25 29) (3 β ,5 α , 25R)-3-[(6'-phenyl-thiocarbamoyl)- β -D-cellobiosyl)oxy]-spirostan-11-one
 252-253°C dec 912 (M+Na) ⁺ HRMS Calc for: C₄₆H₆₇NO₁₄SNa 912.4180
 found 912.4257
- 30 30) (3 β ,5 α , 25R)-3-[(6'-deoxy-6'-[3-(2-fluorophenyl)-ureido]- β -D-cellobiosyl)oxy]-spirostan-11-one
 252-254°C dec 913 (M+Na) ⁺ HRMS Calc for: C₄₆H₆₉FN₂O₁₄Na 914.4550
 found 914.4631

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31) (3 β ,5 α , 25R)-3-[(4',6'-Bis[pyrrolidin-1-yl carbonyl]- β -D-cellobiosyl)oxy]-
spirostan-11-on

>265°C 971 (M+Na) ⁺ C₄₉H₇₆N₂O₁₆ calc. C 60.51; H 8.15; N 2.88
+1.3H₂O found C 60.37; H 8.02; N 3.06

5

32) (3 β ,5 α , 25R)-3-[(4',6'-Bis[morpholin-1-yl carbonyl]- β -D-cellobiosyl)oxy]-
spirostan-11-one

>265°C 1003 (M+Na) ⁺ C₄₉H₇₆N₂O₁₈ calc. C 58.59; H 7.89; N 2.79
+1.3H₂O found C 58.33; H 7.60; N 2.98

10

33) (3 β ,5 α , 25R)-3-[(6'-[2-fluoro-phenylcarbamoyl]- β -D-lactosyl)oxy]-spirostan-11-
one

>265°C 914 (M+Na) ⁺ C₄₈H₆₆NO₁₅ calc. C 60.12; H 7.57; N 1.52
+1.5H₂O found C 60.20; H 7.71; N 1.63

15

34) (3 β ,5 α , 25R)-3-[(4',6'-Bis[3-nitro-phenylcarbamoyl]- β -D-cellobiosyl)oxy]-
spirostan-11-one

247-251°C 1105 (M+Na) ⁺ C₅₃H₇₀N₄O₂₀ calc. C 56.88; H 6.66; N 5.01
+2H₂O found C 56.79; H 6.86; N 5.07

20

35) (3 β ,5 α , 25R)-3-[(6'-deoxy-6'-(2,6-dichloro-benzamido)- β -D-cellobiosyl)oxy]-
spirostan-11-one

>265°C 949 (M+Na) ⁺ C₄₆H₆₅Cl₂NO₁₄ calc. C 59.47; H 6.96; N 1.41
found C 59.61; H 7.07; N 1.51

25

36) (3 β ,5 α , 25R)-3-[(6'-deoxy-6'-benzamido- β -D-cellobiosyl)oxy]-spirostan-11-on

>265°C 880 (M+Na) ⁺ C₄₈H₆₇NO₁₄ calc. C 64.21; H 8.11; N 1.69
found C 64.39; H 7.87; N 1.63

30 37) (3 β ,5 α , 25R)-3-[(6'-deoxy-6'-azido- β -D-cellobiosyl)oxy]-spirostan-11-one

252-254°C dec 802 (M+Na) ⁺ C₃₉H₆₁N₃O₁₃ calc. C 59.38; H 7.92; N 5.33
+0.5H₂O found C 59.41; H 7.81; N 5.13

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- 38) (3 β .5 α . 25R)-3-[(6'-deoxy-6'-phenylacetamido- β -D-cellobiosyl)oxy]-spirostan-11-one
 >265°C 894 (M+Na) ⁺ C₄₇H₈₉NO₁₄ calc. C 64.20; H 8.00; N 1.59
 +0.4 H₂O found C 64.04; H 8.00; N 1.72
- 5
- 39) (3 β .5 α . 25R)-3-[(6'-[α -methoxycarbonyl-benzylcarbamoyl]- β -D-cellobiosyl)oxy]-spirostan-11-one
 >265°C 968 (M+Na) ⁺ C₄₉H₇₁NO₁₇ calc. C 61.27; H 7.62; N 1.46
 +0.8H₂O found C 61.33; H 7.74; N 1.41
- 10 40) (3 β .5 α . 25R)-3-[(6'-Deoxy-6'-(2-fluoro-phenylacetamido)- β -D-cellobiosyl)oxy]-spirostan-11-one
 >265°C 898 (M+Na) ⁺ C₄₈H₈₈FNO₁₄ calc. C 60.58; H 7.74; N 1.54
 +H₂O found C 60.44; H 7.41; N 1.45
- 15 41) (3 β .5 α . 25R)-3-[(4',6'-Bis(2-fluoro-benzylcarbamoyl)- β -D-cellobiosyl)oxy]-spirostan-11-one
 212-213°C 1079 (M+Na) ⁺ HRMS Calc for: C₅₅H₇₄F₂N₂O₁₆Na 914.4550
 found 914.4631
- 20 42) (3 β .5 α . 25R)-3-[(4',6'-Bis[2-thienyl-methylcarbamoyl]- β -D-cellobiosyl)oxy]-spirostan-11-one
 220-222°C 1055 (M+Na) ⁺ C₅₁H₇₂N₂O₁₈S₂ calc. C 58.77; H 7.06; N 2.69
 +0.5H₂O found C 58.76; H 7.38; N 2.66
- 25 43) (3 β .5 α . 25R)-3-[(4',6'-Bis(furan-2-yl-methylcarbamoyl)- β -D-cellobiosyl)oxy]-spirostan-11-one
 244-246°C 1023 (M+Na) ⁺ C₅₁H₇₂N₂O₁₈ calc. C 60.75; H 7.28; N 2.78
 +0.4H₂O found C 60.73; H 7.54; N 2.73

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- 44) (3 β ,5 α , 25R)-3-[(4',6'-Bis[2-ethoxycarbonyl-propylcarbamoyl]- β -D-cellobiosyl)oxy]-spirostan-11-one
 212-214°C 1091 (M+Na) ⁺ C₅₃H₈₄N₂O₂₀ calc. C 58.55; H 7.97; N 2.58
 +1H₂O found C 58.53; H 8.33; N 2.43
- 5
- 45) (3 β ,5 α , 25R)-3-[(4',6'-Bis[ethoxycarbonyl-methylcarbamoyl]- β -D-cellobiosyl)oxy]-spirostan-11-one
 246-248°C 1035 (M+Na) ⁺ HRMS Calc for: C₄₉H₇₈N₂O₂₀Na 1035.4889
 found 1035.4906
- 10 46) (3 β ,5 α , 25R)-3-[(6'-[1-ethoxycarbonyl-2-(2-thienyl)-ethylcarbamoyl]- β -D-cellobiosyl)oxy]-spirostan-11-one
 241-244°C 1002 (M+Na) ⁺ C₄₉H₇₃NO₁₇S calc. C 58.96; H 7.57; N 1.40
 +1H₂O found C 58.98; H 7.71; N 1.46
- 15 47) (3 β ,5 α , 25R)-3-[(6'-[1-ethoxycarbonyl-2-phenyl-ethylcarbamoyl]- β -D-cellobiosyl)oxy]-spirostan-11-one
 241-244°C 996 (M+Na) ⁺ C₅₁H₇₅NO₁₇ calc. C 60.86; H 7.87; N 1.39
 +1.8H₂O found C 60.91; H 7.87; N 1.42
- 20 48) (3 β ,5 α , 25R)-3-[(4',6'-Bis[1-ethoxycarbonyl-2-phenyl-ethylcarbamoyl]- β -D-cellobiosyl)oxy]-spirostan-11-one
 184-185°C 1215 (M+Na) ⁺ C₆₃H₈₈N₂O₂₀ calc. C 62.65; H 7.48; N 2.32
 +0.8H₂O found C 62.75; H 7.82; N 2.23
- 25 49) (3 β ,5 α , 25R)-3-[(4',6'-Bis[2-oxo-propylcarbamoyl]- β -D-cellobiosyl)oxy]-spirostan-11-one
 258-259°C dec 975 (M+Na) ⁺ C₄₇H₇₂N₂O₁₈ calc. C 57.92; H 7.69; N 2.87
 +1.2H₂O found C 57.94; H 8.00; N 2.59
- 30 50) (3 β ,5 α , 12 β ,25R)-3-[(4',6'-Bis[ethoxycarbonyl-methylcarbamoyl]- β -D-cellobiosyl)oxy]-12-hydroxy-spirostan-11-one
 221-222°C dec 1051 (M+Na) ⁺ C₄₉H₇₆N₂O₂₁ calc. C 55.72; H 7.54; N 2.65
 +1.5H₂O found C 55.73; H 7.65; N 2.88

- 51) (3 β .5 α . 25R)-3-[(4',6'-Bis[2-methoxycarbonyl-ethylcarbamoyl]- β -D-cell bi syl)oxy]-spirostan-11-one
 234-238°C 1035 (M+Na) ⁺ C₄₉H₇₈N₂O₂₀ calc. C 57.08; H 7.62; N 2.72
 +1H₂O found C 57.25; H 7.82; N 2.56
- 5 52) (3 β .5 α . 25R)-3-[(4',6'-Bis[tetrahydro-furan-2-yl-methylcarbamoyl]- β -D-cellobiosyl)oxy]-spirostan-11-one
 259-262°C 1031 (M+Na) ⁺ C₅₁H₈₀N₂O₁₈ calc. C 59.42; H 8.06; N 2.72
 +1.2H₂O found C 59.44; H 8.31; N 2.49
- 10 53) (3 β .5 α . 25R)-3-[(4',6'-Bis[3-thienyl-methylcarbamoyl]- β -D-cellobiosyl)oxy]-spirostan-11-one
 228-230°C 1055 (M+Na) ⁺ C₅₁H₇₂N₂O₁₆S₂ calc. C 58.27; H 7.09; N 2.66
 +1H₂O found C 58.31; H 7.22; N 2.63
- 15 54) (3 β .5 α . 25R)-3-[(4',6'-Bis[2-(2-thienyl)-ethylcarbamoyl]- β -D-cellobiosyl)oxy]-spirostan-11-one
 242-245°C 1083 (M+Na) ⁺ C₅₃H₇₈N₂O₁₆S₂ calc. C 58.98; H 7.28; N 2.60
 +1H₂O found C 59.11; H 7.52; N 2.44
- 20 55) (3 β .5 α . 25R)-3-[(4',6'-Bis[3,4-methylenedioxy-benzylcarbamoyl]- β -D-cellobiosyl)oxy]-spirostan-11-one
 209-210°C 1131 (M+Na) ⁺ C₅₇H₇₆N₂O₂₀ calc. C 60.54; H 6.99; N 2.48
 +1.2H₂O found C 60.68; H 7.30; N 2.48
- 25 56) (3 β .5 α . 25R)-3-[(4',6'-Bis[2-trifluoromethyl-benzylcarbamoyl]- β -D-cellobiosyl)oxy]-spirostan-11-one
 230-240°C 1179 (M+Na) ⁺ C₅₇H₇₄F₆N₂O₁₆ calc. C 58.52; H 6.50; N 2.39
 +0.7H₂O found C 58.56; H 6.45; N 2.45
- 30 57) (3 β .5 α . 25R)-3-[(4',6'-Bis[5-methyl-2-thienyl-methylcarbamoyl]- β -D-cellobiosyl)oxy]-spirostan-11-one
 214-215°C 1083 (M+Na) ⁺ C₅₃H₇₈N₂O₁₆S₂ calc. C 59.48; H 7.25; N 2.62
 +0.5H₂O found C 59.46; H 7.29; N 2.73

58) (3 β ,5 α , 25R)-3-[(4',6'-Bis[4-bromo-2-thienyl-methylcarbamoyl]- β -D-cellobiosyl)oxy]-spirostan-11-on

211-213°C 1213 (M+Na) ⁺ C₅₁H₇₀Br₂N₂O₁₈S₂ calc. C 51.37; H 5.87; N 2.41
found C 51.43; H 5.92; N 2.35

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59) (3 β ,5 α , 25R)-3-[(4',6'-Bis[5-bromo-2-thienyl-methylcarbamoyl]- β -D-cellobiosyl)oxy]-spirostan-11-one

226-227°C 1213 (M+Na) ⁺ C₅₁H₇₀Br₂N₂O₁₈S₂ calc. C 51.04; H 5.96; N 2.33
+0.05H₂O found C 51.03; H 6.35; N 2.40

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60) (3 β ,5 α , 25R)-3-[(4',6'-Bis[2-thienylcarbamoyl]- β -D-cellobiosyl)oxy]-spirostan-11-one

261-262°C dec 1027 (M+Na) ⁺ C₄₉H₆₈N₂O₁₆S₂ calc. C 56.52; H 6.97; N 2.69
+2H₂O found C 56.48; H 7.13; N 2.74

15

61) (3 β ,5 α , 12 β ,25R)-3-[(4',6'-Bis[3-thienyl-methylcarbamoyl]- β -D-cellobiosyl)oxy]-12-hydroxy-spirostan-11-one

238-239°C 1071 (M+Na) ⁺ C₅₁H₇₂N₂O₁₇S₂ calc. C 56.91; H 7.02; N 2.60
+1.5H₂O found C 56.85; H 6.86; N 2.65

20

62) (3 β ,5 α , 25R)-3-[(4',6'-Bis[2-methoxycarbonyl-2-methyl-propylcarbamoyl]- β -D-cellobiosyl)oxy]-spirostan-11-one

239-240°C 1091 (M+Na) ⁺ C₅₃H₈₄N₂O₂₀ calc. C 57.88; H 8.01; N 2.55
+1.7H₂O found C 57.83; H 8.11; N 2.66

25

63) (3 β ,5 α , 25R)-3-[(4',6'-Bis[2-oxo-2-(2-thienyl)-ethylcarbamoyl]- β -D-cellobiosyl)oxy]-spirostan-11-one

270-271°C dec 1111 (M+Na) ⁺ C₅₃H₇₂N₂O₁₈S₂ calc. C 57.49; H 6.74; N 2.53
+1H₂O found C 57.37; H 6.73; N 2.57

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64) (3 β ,5 α ,12 β ,25R)-3-[(4',6'-Bis[2-methoxycarbonyl-ethylcarbamoyl]- β -D-cellobiosyl)oxy]-12-hydroxy-spirostan-11-one

232-236°C dec 1051 (M+Na) + C₄₉H₇₈N₂O₂₁ calc. C 55.53; H 7.55; N 2.64
+1.7H₂O found C 55.58; H 7.62; N 2.60

5

65) (3 β ,5 α ,25R)-3-[(4',6'-Bis[thiazol-2-yl-methylcarbamoyl]- β -D-cellobiosyl)oxy]-spirostan-11-one

251-252°C 1057 (M+Na) + C₄₇H₇₀N₄O₁₆S₂ calc. C 55.59; H 6.91; N 5.29
+1.3H₂O found C 55.74; H 7.16; N 4.97

10

66) (3 β ,5 α ,25R)-3-[(4',6'-Bis[benzylcarbamoyl]- β -D-cellobiosyl)oxy]-spirostan-11-one

254-255°C dec 1043 (M+Na) + C₅₅H₇₆N₂O₁₈ calc. C 63.02; H 7.60; N 2.67
+1.5H₂O found C 63.00; H 7.61; N 2.79

15

67) (3 β ,5 α ,25R)-3-[(4',6'-Bis[5-trifluoromethyl-benzothiazol-2-yl-methylcarbamoyl]- β -D-cellobiosyl)oxy]-spirostan-11-one

>265°C 1293 (M+Na) + C₅₉H₇₂F₆N₄O₁₆S₂ calc. C 54.35; H 5.84; N 4.30
+1.8H₂O found C 54.16; H 5.96; N 4.28

20

68) (3 β ,5 α ,25R)-3-[(4',6'-Bis[4-methoxy-benzylcarbamoyl]- β -D-cellobiosyl)oxy]-spirostan-11-one

222-224°C 1103 (M+Na) + C₅₇H₈₀N₂O₁₈ calc. C 60.50; H 7.62; N 2.48
+2.8H₂O found C 60.38; H 7.51; N 2.63

25

69) (3 β ,5 α ,25R)-3-[(4',6'-Bis[2-(2-fluorophenyl)ethylcarbamoyl]- β -D-cellobiosyl)oxy]-spirostan-11-one

221-223°C 1107 (M+Na) + C₅₇H₇₈F₂N₂O₁₆ calc. C 61.35; H 7.35; N 2.51
+1.7H₂O found C 61.37; H 7.36; N 2.69

- 70) (3 β ,5 α ,12 β ,25R)-3-[(4',6'-Bis[thiazol-2-yl-methylcarbamoyl]- β -D-cellobiosyl)oxy]-12-hydroxy-spirostan-11-one
 229-232°C dec 1073 (M+Na) ⁺ C₄₉H₇₀N₄O₁₇S₂ calc. C 53.95; H 6.87; N 5.14
 +2.2H₂O found C 53.90; H 6.85; N 5.03
- 5
- 71) (3 β ,5 α ,25R)-3-[(4',6'-Bis[3-methyl-isoxazol-5-yl-methylcarbamoyl]- β -D-cellobiosyl)oxy]-spirostan-11-one
 242-244°C 1053 (M+Na) ⁺ C₅₁H₇₄N₄O₁₈ calc. C 57.21; H 7.38; N 5.23
 +2.2H₂O found C 57.06; H 7.60; N 5.41
- 10
- 72) (3 β ,5 α ,25R)-3-[(4',6'-Bis[2-methoxycarbonyl-butylcarbamoyl]- β -D-cellobiosyl)oxy]-spirostan-11-one
 216-217°C 1091 (M+Na) ⁺ C₅₃H₈₄N₂O₂₀ calc. C 57.57; H 8.02; N 2.53
 +2H₂O found C 57.45; H 8.31; N 2.83
- 15
- 73) (3 β ,5 α ,25R)-3-[(6'-bromo-6'-deoxy- β -D-cellobiosyl)oxy]-spirostan-11-one
 169-170°C (dec) 828 (M+Na) ⁺ C₃₉H₆₁BrO₁₃ calc. C 55.21; H 7.65
 +1.7H₂O found C 55.59; H 8.03
- 20
- 74) (3 β ,5 α ,25R)-3-[(6'-deoxy- β -D-cellobiosyl)oxy]-spirostan-11-one
 257-260°C (dec) 760 (M+Na) ⁺ C₃₉H₆₂O₁₃ calc. C 59.62; H 8.62
 +2.6H₂O found C 59.58; H 8.97
- 25
- 75) (3 β ,5 α ,25R)-3-[(6'-deoxy-4'-[2-fluoro-phenylcarbamoyl]- β -D-cellobiosyl)oxy]-spirostan-11-one
 262-265°C (dec) 898 (M+Na) ⁺ C₄₆H₆₈FNO₁₄ calc. C 62.43; H 7.63; N 1.58
 +0.5H₂O found C 62.13; H 8.02; N 1.89
- 30
- 76) (3 β ,5 α ,11 β ,25R)-3-[(4',6'-Bis[2-fluoro-phenylcarbamoyl]- β -D-cellobiosyl)oxy]-11-hydroxy-spirostane
 268-270°C 1053 (M+Na) ⁺ HRMS calc for: C₅₃H₇₂F₂N₂O₁₆ 1053.4748
 found: 1053.47628

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77) (3 β ,5 α ,25R)-3-[(4',6'-Bis[pyridin-3-yl-methylcarbamoyl]- β -D-cellobiosyl)oxy]-spirostan-11-on

234-237°C 1045 (M+Na)⁺ C₅₃H₇₄N₄O₁₆ calc. C 59.09; H 7.49; N 5.20
+3H₂O found C 59.16; H 7.83; N 5.26

5

Example 78

(3 β ,5 α ,25R)-3-[(4',6'-bis[2-fluoro-phenylcarbamoyl]- β -D-cellobiosyl)oxy]-spirostan-11-one

DEACETYLATION USING POTASSIUM CYANIDE

- 10 To a solution of (3 β ,5 α ,25R)-3-[(4',6'-bis-[2-fluoro-phenylcarbamoyl]-pentaacetyl- β -D-cellobiosyl)oxy]-spirostan-11-one (0.48g, 0.39 mmol) in tetrahydrofuran (3 mL) and methanol (3 mL), potassium cyanide (38 mg, 0.58 mmol) was added. The reaction was stirred at room temperature under nitrogen atmosphere for 4 hours. Upon completion, methanol was added and the reaction mixture was concentrated
- 15 in *vacuo*. Methanol, then water was added to the residual material, causing a precipitate to form. The precipitate was filtered, washed with water and dried in a vacuum oven. The crude product was purified via flash chromatography (2% methanol: chloroform). The isolated material was concentrated in vacuo, then triturated with methanol and water. The product was then filtered, washed with water
- 20 and oven-dried to afford 33 mg (8.3 % yield) of the title compound. ¹HNMR (250 MHz; DMSO-d₆) δ 9.4 (s, 1H); 9.3 (s, 1H); 7.7-7.05 (m, 8H); 5.5 (d, 1H, J=7 Hz); 5.45 (d, 1H, J=7 Hz); 5.0 (d, 1H, J=7 Hz); 4.7-2.9 (m, 20H); 2.55-1.1 (m, 25H); 0.95 (s, 3H); 0.85 (d, 3H, J=8 Hz); 0.7 (d, 3H, J=8 Hz); 0.6 (s, 3H). FAB MS: 1051 (M+Na)⁺; Analysis calculated for C₅₃H₇₀F₂N₂O₁₆·1 H₂O: C 60.79, H 6.93, N 2.67;
- 25 found: C 60.63, H 6.72, N 2.83; m.p. > 265°C.

Examples 79-83

The following compounds were prepared from the appropriate starting material in an analogous manner using the above procedures.

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Exempl)	Name	m.p.	M.S.	formula	el mental analysis
=====					
79)	<u>(3β,5α,25R)-3-[(4",6"-Bis[2,4-difluoro-phenylcarbamoyl]-β-D-cellobiosyl)oxy]-</u>				
5	<u>spirostan-11-one</u>				
		>265°C	1087(M+Na) ⁺	C ₅₃ H ₆₈ F ₄ N ₂ O ₁₆	calc. C 58.77; H 6.51; N 2.59
		(dec)		+1H ₂ O	found C 58.79; H 6.47; N 2.49
80)	<u>(3β,5α,25R)-3-[(4",6"-Bis[2,6-dichloro-phenylcarbamoyl]-β-D-cellobiosyl)oxy]-</u>				
10	<u>spirostan-11-one</u>				
		262-264°C	1153(M+Na) ⁺	HRMS calc. for C ₅₃ H ₆₈ Cl ₄ N ₂ O ₁₆ Na	1151.3221
					found 1151.3216
81)	<u>(3β,5α,25R)-3-[(4",6"-Bis[2-chloro-phenylcarbamoyl]-β-D-cellobiosyl)oxy]-</u>				
15	<u>spirostan-11-one</u>				
		247-250°	1085(M+Na) ⁺	C ₅₃ H ₇₀ Cl ₂ N ₂ O ₁₆	calc. C 58.94; H 6.72; N 2.59
				+1.0 H ₂ O	found C 58.70; H 6.65; N 2.70
82)	<u>(3β,5α,25R)-3-[(4",6"-Bis[2-methyl-phenylcarbamoyl]-β-D-cellobiosyl)oxy]-</u>				
20	<u>spirostan-11-one</u>				
		249-250°	1043(M+Na) ⁺	C ₅₅ H ₇₈ N ₂ O ₁₆	calc. C 63.57; H 7.56; N 2.69
				+1.0 H ₂ O	found C 63.39; H 7.46; N 2.99
83)	<u>(3β,5α,25R)-3-[(4",6"-Bis[phenylcarbamoyl]-β-D-cellobiosyl)oxy]-spirostan-11-</u>				
25	<u>one</u>				
		>265°C	1014(M+Na) ⁺	C ₅₃ H ₇₂ N ₂ O ₁₆	calc. C 62.40; H 7.41; N 2.75
				+1.5 H ₂ O	found C 62.48; H 7.22; N 3.06

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Exempl 84(3 β ,5 α ,25R)-3-[(6',6"-Bis[2,4-dichloro-ph nylcarbamoyl]- β -D-cellobiosyl)oxy]-spirostan-11-oneCARBAMOYLATION

- 5 A mixture of (3 β ,5 α ,25R)-3-[(β -D-cellobiosyl)oxy]spirostan-11-one (0.50 g, 0.66 mmol), pyridine (3 mL) and 4A molecular sieves (0.50 g) was stirred for 10 minutes at room temperature under nitrogen atmosphere. The reaction was then cooled to -40°C and 2,4-dichlorophenyl isocyanate was added. The reaction mixture was gradually warmed to room temperature and stirred for 1.5 hours. Upon
- 10 completion, the reaction was quenched with methanol. The quenched mixture was concentrated in vacuo twice with toluene in order to remove the pyridine. The crude material was purified via flash chromatography (2% to 10% methanol: chloroform). The first product isolated was the 6',6"-dicarbamate which was concentrated in vacuo, triturated with methanol/water, filtered and washed with water to afford 230
- 15 mg (40 % yield) of the title compound. FAB MS: 1129 (M+H)⁺; m.p. 182°C.

Example 85(3 β ,5 α ,25R)-3-[(6'-[2,4-dichloro-phenylcarbamoyl]- β -D-cellobiosyl)oxy]-spirostan-11-one

- Further elution of the column in Example 84 gave the 6"-monocarbamate, 35
- 20 mg (6% yield). FAB MS: 942 (M+H)⁺; Analysis calculated for C₄₆H₈₅Cl₂NO₁₅+0.5 H₂O: C 58.04, H 6.99, N 1.47; found: C 58.01, H 6.86, N 1.48; m.p. 241-242°C.

Examples 86-119

- The following compounds were prepared from the appropriate starting material and isocyanate or acid chloride in an analogous manner using the above
- 25 procedures.

Example) Name

m.p. M.S. formula elemental analysis

=====

- 86) (3 β ,5 α ,25R)-3-[(6',6"-Bis[4-chloro-phenylcarbamoyl]- β -D-cellobiosyl)oxy]-
- 30 spirostan-11-one
- 233°C 1061(M+H)⁺ C₅₃H₇₀Cl₂N₂O₁₆ calc. C 58.74; H 6.73; N 2.58
(dec) +1.2 H₂O found C 58.55; H 6.54; N 2.66)

- 87) (3 β ,5 α ,25R)-3-[(6',6"-Bis[2,6-dichloro-phenylcarbamoyl]- β -D-cellobiosyl)oxy]-spirostan-11-one
 260-265°C 1131(M+H)⁺ HRMS calc. for C₅₃H₈₈N₂Cl₄O₁₆Na 1151.3221
 (dec) found 1151.3206
- 5
- 88) (3 β ,5 α ,25R)-3-[(6',6"-Bis[benzylcarbamoyl]- β -D-cellobiosyl)oxy]-spirostan-11-one
 164-165°C 1043(M+Na)⁺ HRMS calc. for C₅₅H₇₆N₂O₁₆Na 1043.5093
 found: 1043.5178
- 10
- 89) (3 β ,5 α ,25R)-3-[(6',6"-Bis[2-fluoro-phenylcarbamoyl]- β -D-cellobiosyl)oxy]-spirostan-11-one
 249°C 1051(M+Na)⁺ C₅₃H₇₀F₂N₂O₁₆ calc. C 59.76; H 7.02; N 2.63
 +2H₂O found C 59.91; H 6.93; N 2.57
- 15
- 90) (3 β ,5 α ,25R)-3-[(6',6"-Bis[2-chloro-phenylcarbamoyl]- β -D-cellobiosyl)oxy]-spirostan-11-one
 >265°C 1083(M+Na)⁺ C₅₃H₇₀Cl₂N₂O₁₆ calc. C 58.94; H 6.72; N 2.59
 +1.0 H₂O found C 58.66; H 6.58; N 2.74
- 20
- 91) (3 β ,5 α ,25R)-3-[(6',6"-Bis[2-methyl-phenylcarbamoyl]- β -D-cellobiosyl)oxy]-spirostan-11-one
 205-208°C 1043(M+Na)⁺ C₅₅H₇₈N₂O₁₆ calc. C 63.57; H 7.56; N 2.69
 +1.0 H₂O found C 63.51; H 7.41; N 2.77
- 25
- 92) (3 β ,5 α ,25R)-3-[(6"-[4-chloro-phenylcarbamoyl]- β -D-cellobiosyl)oxy]-spirostan-11-one
 >265°C 930(M+Na)⁺ C₄₆H₆₆ClNO₁₅ calc. C 60.82; H 7.32; N 1.54
 found C 60.71; H 7.24; N 1.33
- 30

- 93) (3 β ,5 α ,25R)-3-[(6'-[4-chloro-phenylcarbamoyl]- β -D-cellobiosyl)oxy]-spirostan-11-one
 >255°C 908(M+H)⁺ C₄₆H₈₈ClNO₁₅ calc. C 59.63; H 7.40; N 1.51
 930(M+Na)⁺ +1.0 H₂O found C 59.54; H 7.24; N 1.33
- 5 94) (3 β ,5 α ,25R)-3-[(6'-[2,6-dichloro-phenylcarbamoyl]- β -D-cellobiosyl)oxy]-spirostan-11-one
 253-254°C 964(M+Na)⁺ C₄₆H₈₅Cl₂NO₁₅ calc. C 58.59; H 6.59; N 1.48
 found C 58.55; H 6.84; N 1.29
- 10 95) (3 β ,5 α ,25R)-3-[(6'-[2,4-difluoro-phenylcarbamoyl]- β -D-cellobiosyl)oxy]-spirostan-11-one
 >265°C 910(M+H)⁺ C₄₆H₈₅F₂NO₁₅ calc. C 60.71; H 7.20; N 1.54
 found C 60.36; H 6.89; N 1.28
- 15 96) (3 β ,5 α ,25R)-3-[(6'-[2-fluoro-phenylcarbamoyl]- β -D-cellobiosyl)oxy]-spirostan-11-one
 >265°C 914(M+Na)⁺ C₄₆H₈₅FNO₁₅ calc. C 60.71; H 7.53; N 1.54
 +1.0 H₂O found C 60.92; H 7.45; N 1.76
- 20 97) (3 β ,5 α ,25R)-3-[(6'-[benzylcarbamoyl]- β -D-cellobiosyl)oxy]-spirostan-11-one
 >260°C 910(M+Na)⁺ HRMS calc. for C₄₇H₈₉NO₁₅Na 910.4565
 found 910.4633
- 25 98) (3 β ,5 α ,25R)-3-[(6'-[2-chloro-phenylcarbamoyl]- β -D-cellobiosyl)oxy]-spirostan-11-one
 264-265°C 930(M+Na)⁺ C₄₆H₈₈ClNO₁₅ calc. C 60.69; H 7.33; N 1.53
 +1.0 H₂O found C 60.40; H 7.31; N 1.70
- 30 99) (3 β ,5 α ,25R)-3-[(6'-[2-methyl-phenylcarbamoyl]- β -D-cellobiosyl)oxy]-spirostan-11-one
 254-256°C 910(M+Na)⁺ C₄₇H₈₉NO₁₅ calc. C 62.30; H 7.90; N 1.54
 +1.0 H₂O found C 62.17; H 7.87; N 1.61

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- 100) (3 β ,5 α ,25R)-3-[(6',6"-Bis[2,6-dichloro-benzoyl]- β -D-lactosyl)oxy]-spirostan-11-one
 255°C 1099(M+H)⁺ C₅₃H₈₈Cl₄O₁₆ calc. C 57.82; H 6.04
 (dec) 1121(M+Na)⁺ found C 57.49; H 6.01
- 5
- 101) (3 β ,5 α ,25R)-3-[(6',6"-Bis[4-fluoro-benzoyl]- β -D-cellobiosyl)oxy]-spirostan-11-one
 173-174°C 999(M+H)⁺ C₅₃H₈₈F₂O₁₆ calc. C 62.37; H 6.95
 +1.2 H₂O found C 62.13; H 6.92
- 10 102) (3 β ,5 α ,25R)-3-[(6',6"-Bis[furan-2-carbonyl]- β -D-cellobiosyl)oxy]-spirostan-11-one
 248-250°C 943(M+H)⁺ C₄₉H₆₆O₁₈ calc. C 61.53; H 7.11
 (dec) 964(M+Na)⁺ +0.75 H₂O found C 61.45; H 7.01
- 15 103) (3 β ,5 α ,25R)-3-[(6',6"-Bis[2,6-dimethoxy-benzoyl]- β -D-cellobiosyl)oxy]-spirostan-11-one
 257-258°C 1105(M+Na)⁺ C₅₇H₇₈O₂₀ calc. C 61.17; H 7.38
 +2.0 H₂O found C 61.10; H 7.19
- 20 104) (3 β ,5 α ,25R)-3-[(6'-[2,6-dichloro-benzoyl]- β -D-cellobiosyl)oxy]-spirostan-11-one
 269-271°C 926(M+H)⁺ C₄₆H₆₄Cl₂O₁₅ calc. C 59.54; H 6.95
 (dec) found C 59.39; H 6.74
- 105) (3 β ,5 α ,25R)-3-[(6'-[2,6-difluoro-benzoyl]- β -D-cellobiosyl)oxy]-spirostan-11-one
 25 272-274°C 895(M+H)⁺ C₄₆H₆₄F₂O₁₅ calc. C 61.73; H 7.21
 917(M+Na)⁺ found C 61.58; H 6.94
- 106) (3 β ,5 α ,25R)-3-[(6'-[4-chloro-benzoyl]- β -D-cellobiosyl)oxy]-spirostan-11-one
 254-255°C 893(M+H)⁺ C₄₆H₆₅ClO₁₅ calc. C 61.84; H 7.33
 30 found C 61.47; H 7.09

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- 107) (3 β ,5 α ,25R)-3-[(6"-[4-fluoro-benzoyl]- β -D-cellobiosyl)oxy]-spirostan-11-one
 262-265°C 877(M+H)⁺ C₄₈H₆₅FO₁₅ calc. C 62.36; H 7.51
 +0.5 H₂O found C 62.55; H 7.26
- 5 108) (3 β ,5 α ,25R)-3-[(6"-[2,6-dichloro-benzoyl]- β -D-lactosyl)oxy]-spirostan-11-one
 247-248°C 927(M+H)⁺ C₄₆H₆₄Cl₂O₁₅ calc. C 59.54; H 6.95
 949(M+Na)⁺ found C 59.35; H 6.70
- 109) (3 β ,5 α ,25R)-3-[(6"-[furan-2-carbonyl]- β -D-cellobiosyl)oxy]-spirostan-11-one
 10 >265°C 849(M+H)⁺ C₄₄H₆₄O₁₆ calc. C 62.25; H 7.60
 found C 62.22; H 7.61
- 110) (3 β ,5 α ,25R)-3-[(6"-[2,4-difluoro-benzoyl]- β -D-cellobiosyl)oxy]-spirostan-11-one
 261-262°C 895(M+H)⁺ C₄₆H₆₄F₂O₁₅ calc. C 60.75; H 7.27
 15 +0.8 H₂O found C 60.74; H 6.89
- 111) (3 β ,5 α ,25R)-3-[(6"-[2,6-dimethoxy-benzoyl]- β -D-cellobiosyl)oxy]-spirostan-11-one
 263-265°C 941(M+Na)⁺ C₄₈H₇₀O₁₇ calc. C 62.73; H 7.68
 20 found C 62.83; H 7.58
- 112) (3 β ,5 α ,25R)-3-[(6',6"-Bis[2,6-difluoro-benzoyl]- β -D-cellobiosyl)oxy]-spirostan-11-one
 230°C 1035(M+H)⁺ C₅₇H₆₆F₄O₁₆ calc. C 59.74; H 6.55
 25 (dec) 1057(M+Na)⁺ +1.5 H₂O found C 59.70; H 6.45
- 113) (3 β ,5 α ,25R)-3-[(6',6"-Bis[2-methoxycarbonyl-phenylcarbamoyl]- β -D-cellobiosyl)oxy]-spirostan-11-one
 249-251°C dec 1131 (M+Na)⁺ C₅₇H₇₆N₂O₂₀ calc. C 59.78; H 7.04; N 2.45
 30 +2H₂O found C 59.54; H 7.00; N 2.55

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Preparation A1

(3 β ,5 α ,25R)-3-[(4',6'-Bis[2-oxo-propylcarbamoyl]-2',2',3',3',6'-penta-acetyl- β -D-cellobiosyl)oxy]-spirostan-11-one

PCC OXIDATION

5 Pyridinium chlorochromate (1.0 g, 4.7 mmol) was added to a solution of (3 β ,5 α ,25R)-3-[(4',6'-bis[2-hydroxy-propylcarbamoyl]-2',2',3',3',6'-penta-acetyl- β -D-cellobiosyl)oxy]-spirostan-11-one (482 mg, 0.413 mmol) in methylene chloride (10 mL) containing dry Celite filter aid (1.5 g). After 5h at room temperature an additional 350 mg of pyridinium chlorochromate was added and the reaction stirred
 10 overnight. The mixture was diluted with ether (20 mL) and the mixture was filtered through silica gel (eluting with ethyl acetate). The filtrate was concentrated and the residue was purified by flash chromatography (3% methanol/methylene chloride) to give 400 mg of the title compound as a colorless foam. ¹H NMR (250 MHz, CDCl₃) δ 6.5 (bt, 1H, J = 6.0 Hz); 6.0 (bt, 1H, J = 6.0 Hz); 5.15 (m, 2H); 4.9 m (3H);
 15 4.6-3.5 (m, 12H); 3.4 (dd, 1H, J = 8.0, 8.0 Hz); 2.45 (m, 1H); 2.22 (s, 2H); 2.2 (s, 3H); 2.12 (s, 3H); 2.08 (s, 6H); 2.05 (s, 6H); 2.0 (s, 3H); 2.0-1.0 (m, 26H); 1.0 (s, 3H); 0.9 (d, 3H, J = 7.0 Hz); 0.75 (d, 3H, J = 7.0 Hz); 0.65 (s, 3H).

In an analogous manner, the following compound, Preparation A2 was prepared from the appropriate starting material using the above general procedure.

20

Preparation A2

(3 β ,5 α ,25R)-3-[(4',6'-Bis[2-oxo-2-(2-thienyl)-ethylcarbamoyl]-2',2',3',3',6'-penta-acetyl- β -D-cellobiosyl)oxy]-spirostan-11-one

Preparation B1

25 (3 β ,5 α , 25R)-3-[(4'-[2-fluoro-phenylcarbamoyl]-6'-triisopropylsilyl- β -D-cellobiosyl)oxy]-spirostan-11-one

DEACYLATION OF SILYL SUBSTITUTED COMPOUNDS

Sodium methoxide (21 mg) was added to a solution of (3 β ,5 α ,25R)-3-[(4'-[2-fluoro-phenylcarbamoyl]-2',2',3',3',6'-pentaacetyl-6'-triisopropylsilyl- β -D-cellobiosyl)oxy]-spirostan-11-one (668 mg, 0.53 mmol) in THF (1 mL) and methanol (2 mL). After 1h, the mixture was concentrated and the residue was purified by flash chromatography (1-3% methanol/chloroform) to afford 322 mg product as a colorless foam (58%). ¹H NMR (250 MHz, CDCl₃) δ 8.0 (bs, 1H); 7.05 (m, 4H);

30

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4.88 (dd, 1H J = 9.0, 9.0 Hz); 4.48 (m, 3H); 4.3 (s, 1H); 3.9-3.3 (m, 16H); 2.8 (t, 1H, J = 6.0 Hz); 2.55 (d, 1H, J = 1.5 Hz); 2.5 (m, 1H); 2.25 (s, 2H); 2.1-1.0 (m, 46H); 0.9 (d, 3H, J = 7.0 Hz); 0.8 (d, 3H, J = 7.0 Hz); 0.7 (s, 3H).

Preparation C1

5 (3 β ,5 α ,25R)-3-[(6',6"-Bis-[phenylcarbamoyl]-pentaacetyl- β -D-cellobiosyl)oxy]-spirostan-11-one

CARBAMOYLATION or ACYLATION

A mixture of (3 β ,5 α ,25R)-3-[(2',2",3',3",4"-pentaacetyl- β -D-cellobiosyl)oxy] spirostan-11-one (0.50 g, 0.52 mmol), methylene chloride (5 mL), triethylamine
 10 (0.50mL, 3.62 mmol) and diethylaminopyridine (0.10 g) was cooled to 0°C under nitrogen atmosphere. Phenyl isocyanate (0.34 mL, 3.12 mmol) was added and the reaction was stirred at 0°C for 15 minutes, then at room temperature for 30 minutes. Upon completion, the reaction was quenched with methanol. The quenched mixture was diluted with ethyl acetate, washed with water (1x), 1N hydrochloric acid solution
 15 (3x), saturated sodium bicarbonate solution (2x) and brine (1x), dried over sodium sulfate, filtered and concentrated in vacuo. The residual material was triturated with hexanes, filtered, washed with hexanes and dried to afford >100% yield of the crude titled compound. ¹HNMR (250MHz, CDCl₃) δ 7.55-7.0 (m, 10H); 6.85 (s, 1H); 6.65 (s, 1H); 5.3-3.52 (m, 17H); 3.45 (t, 1H, J=10 Hz); 2.5-1.05 (m, 40H); 1.0 (s, 3H); 0.95 (d,
 20 3H, J=8 Hz); 0.8 (d, 3H, J=8 Hz); 0.7 (s, 3H).

In an analogous manner the following compounds, Preparations C2-C30, were prepared from the appropriate starting material using the above general procedure.

Preparation C2

25 (3 β ,5 α ,25R)-3-[(6',6"-Bis[2,4-difluoro-phenylcarbamoyl]-pentaacetyl- β -D-cellobiosyl)-oxy]spirostan-11-one

Preparation C3

30 (3 β ,5 α ,25R)-3-[(6"-[2,4-difluoro-phenylcarbamoyl]-pentaacetyl- β -D-cellobiosyl)oxy]spirostan-11-one

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Preparation C4

(3 β ,5 α ,25R)-3-[(4',6'-Bis[2,4-difluoro-phenylcarbamoyl]-pentaacetyl- β -D-cellobiosyl)oxy]spirostan-11-one

5

Preparation C5

(3 β ,5 α ,25R)-3-[(4',6'-Bis[2,6-dichloro-phenylcarbamoyl]-pentaacetyl- β -D-cellobiosyl)oxy]spirostan-11-one

Preparation C6

10

(3 β ,5 α ,25R)-3-[(4',6'-Bis[2-chloro-phenylcarbamoyl]-pentaacetyl- β -D-cellobiosyl)oxy]spirostan-11-one

Preparation C7

15

(3 β ,5 α ,25R)-3-[(4',6'-Bis[2-methyl-phenylcarbamoyl]-pentaacetyl- β -D-cellobiosyl)oxy]spirostan-11-one

Preparation C8

20

(3 β ,5 α ,25R)-3-[(4',6'-Bis[phenylcarbamoyl]-pentaacetyl- β -D-cellobiosyl)oxy]spirostan-11-one

Preparation C9

(3 β ,5 α ,25R)-3-[(4',6'-Bis[2-methoxy-phenylcarbamoyl]-pentaacetyl- β -D-cellobiosyl)oxy]spirostan-11-one

25

Preparation C10

(3 β ,5 α ,25R)-3-[(6'-[2-methoxy-phenylcarbamoyl]-hexaacetyl- β -D-cellobiosyl)oxy]spirostan-11-one

Preparation C11

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(3 β ,5 α ,25R)-3-[(4'-[2-fluoro-phenylcarbamoyl]-hexaacetyl- β -D-cellobiosyl)oxy]spirostan-11-one

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Preparation C12

(3 β ,5 α ,25R)-3-[(4',6'-Bis[2,6-dimethyl-phenylcarbamoyl]-
pentaacetyl- β -D-cellobiosyl)oxy]spirostan-11-one

5

Preparation C13

(3 β ,5 α ,25R)-3-[(4',6'-Bis[2,5-difluoro-phenylcarbamoyl]-
pentaacetyl- β -D-cellobiosyl)oxy]spirostan-11-one

Preparation C14

10

(3 β ,5 α ,12 β ,25R)-3-[(4',6'-Bis[2-fluoro-phenylcarbamoyl]-
pentaacetyl- β -D-cellobiosyl)oxy]12-acetoxy-spirostan-11-one

Preparation C15

15

(3 β ,5 α , 25R)-3-[(4',6'-Bis[2-methoxycarbonyl-phenylcarbamoyl]-
2',2'',3',3'',6'-penta-acetyl- β -D-cellobiosyl)oxy]-spirostan-11-one

Preparation C16

20

(3 β ,5 α , 25R)-3-[(6'-[4-phenoxy-phenylcarbamoyl]-2',2'',3',3'',4',6'-hexa-acetyl
- β -D-cellobiosyl)oxy]-spirostan-11-one

Preparation C17

(3 β ,5 α , 25R)-3-[(4',6'-Bis[allylcarbamoyl]-2',2'',3',3'',6'-penta-acetyl
- β -D-cellobiosyl)oxy]-spirostan-11-one

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Preparation C18

(3 β ,5 α , 25R)-3-[(4',6'-Bis[3,5-dimethoxy-phenylcarbamoyl]
-2',2'',3',3'',6'-penta-acetyl- β -D-cellobiosyl)oxy]-spirostan-11-one

Preparation C19

30

(3 β ,5 α , 25R)-3-[(6'-acetamido-6'-deoxy-4'-[2-fluoro-phenylcarbamoyl]-
2',2'',3',3'',4',6'-hexa-acetyl- β -D-cellobiosyl)oxy]-spirostan-11-one

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Preparation C20

(3 β ,5 α , 25R)-3-[(6'-acetamido-6'-deoxy-2',2',3',3',4',6'-hexa-acetyl- β -D-cellobiosyl)oxy]-spirostan-11-one

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Preparation C21

(3 β ,5 α , 25R)-3-[(6'-deoxy-6'-[3-(2-fluoro-phenyl)-ureido]-2',2',3',3',4',6'-hexa-acetyl- β -D-cellobiosyl)oxy]-spirostan-11-one

Preparation C22

10 (3 β ,5 α , 25R)-3-[(6'-[2-fluoro-phenylcarbamoyl]-2',2',3',3',4',6'-hexa-acetyl- β -D-lactosyl)oxy]-spirostan-11-one

Preparation C23

15 (3 β ,5 α , 25R)-3-[(4',6'-Bis[3-nitro-phenylcarbamoyl]-2',2',3',3',6'-penta-acetyl- β -D-cellobiosyl)oxy]-spirostan-11-one

Preparation C24

20 (3 β ,5 α , 25R)-3-[(6'-deoxy-6'-(2,6-dichloro-benzamido)-2',2',3',3',4',6'-hexa-acetyl- β -D-cellobiosyl)oxy]-spirostan-11-one

Preparation C25

(3 β ,5 α , 25R)-3-[(6'-deoxy-6'-benzamido-2',2',3',3',4',6'-hexa-acetyl- β -D-cellobiosyl)oxy]-spirostan-11-one

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Preparation C26

(3 β ,5 α , 25R)-3-[(6'-deoxy-6'-(phenylacetamido)-2',2',3',3',4',6'-hexa-acetyl- β -D-cellobiosyl)oxy]-spirostan-11-one

Preparation C27

30 (3 β ,5 α , 25R)-3-[(6'-Deoxy-6'-(2-fluoro-phenylacetamido)-2',2',3',3',4',6'-hexa-acetyl- β -D-cellobiosyl)oxy]-spirostan-11-one

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Preparation C28

(3 β ,5 α , 25R)-3-[(4',6'-Bis[2-thienylcarbamoyl]-2',2'',3',3',6'-penta-acetyl- β -D-cellobiosyl)oxy]-spirostan-11-one

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Preparation C29

(3 β ,5 α , 25R)-3-[(6'-deoxy-4'-[2-fluoro-phenylcarbamoyl]-2',2'',3',3',6'-pentaacetyl- β -D-cellobiosyl)oxy]-spirostan-11-one

Preparation C30

10

(3 β ,5 α ,11 β ,25R)-3-[(4',6'-Bis[2-fluoro-phenylcarbamoyl]-2',2'',3',3',6'-pentaacetyl- β -D-cellobiosyl)oxy]-11-acetoxy-spirostane

Preparation D1

15

(3 β ,5 α ,25R)-3-[(4',6'-Bis[4-hydroxy-butylcarbamoyl]-pentaacetyl- β -D-cellobiosyl)oxy]spirostan-11-one

CARBAMOYLATION USING CARBONYL DIMIDAZOLE

A mixture of (3 β ,5 α ,25R)-3-[(2',2'',3',3',6'-pentaacetyl- β -D-cellobiosyl)oxy]spirostan-11-one (1.0g, 1.04 mmol), carbonyl diimidazole (0.42 g, 2.6 mmol), and diisopropylethyl amine (0.9 mL, 5.2 mmol) in dichloroethane (7 mL) was stirred at room temperature for 2 hours; 4-hydroxybutylamine (0.24 mL, 2.6 mmol) was added and the mixture was stirred for 5 hours at room temperature. The mixture was diluted with ethyl acetate and washed with 1N hydrochloric acid (2X), saturated sodium bicarbonate solution (1x) and brine (1x), dried over sodium sulfate, filtered and concentrated in vacuo. The residue was purified by flash chromatography (1-3% methanol/chloroform) to afford 450 mg (37%) product as a colorless solid. ¹H NMR (250 MHz, CDCl₃) δ 7.2 (bt, 2H); 5.1 (m, 2H), 4.8 (m, 2H); 4.55 (m, 1H); 4.3 (m, 2H); 4.1 (m, 1H); 3.8 (m, 2H); 3.6 (m, 1H); 3.4 (m, 4H); 3.2 (m, 1H); 2.9 (m, 4H); 2.5 (s, 2H); 2.3 (m, 2H); 2.0-1.0 (m, 52H); 0.9 (s, 3H); 0.85 (d, 3H, J=7Hz); 0.7 (d, 3H, J=7Hz); 0.6 (s, 3H).

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In an analogous manner, the following compounds, Preparation D2-D38 were prepared from the appropriate starting material using the above general procedure.

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Preparation D2

(3 β ,5 α , 25R)-3-[(4',6'-Bis[phenyl-thiocarbamoyl]-2',2',3',3',6'-penta-acetyl- β -D-cellobiosyl)oxy]-spirostan-11-one

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Preparation D3

(3 β ,5 α , 25R)-3-[(6'-phenyl-thiocarbamoyl -2',2',3',3',6'-penta-acetyl- β -D-cellobiosyl)oxy]-spirostan-11-one

Preparation D4

10 (3 β ,5 α , 25R)-3-[(4',6'-Bis[pyrrolidin-1-ylcarbamoyl]-2',2',3',3',6'-penta-acetyl- β -D-cellobiosyl)oxy]-spirostan-11-one

Preparation D5

15 (3 β ,5 α , 25R)-3-[(4',6'-Bis[morpholin-1-ylcarbamoyl]-2',2',3',3',6'-penta-acetyl- β -D-cellobiosyl)oxy]-spirostan-11-one

Preparation D6

20 (3 β ,5 α , 25R)-3-[(6'-[α -methoxycarbonyl-benzyl carbamoyl]-2',2',3',3',6'-penta-acetyl- β -D-cellobiosyl)oxy]-spirostan-11-one

Preparation D7

(3 β ,5 α , 25R)-3-[(4',6'-Bis[2-fluoro-benzylcarbamoyl]-2',2',3',3',6'-penta-acetyl- β -D-cellobiosyl)oxy]-spirostan-11-one

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Preparation D8

(3 β ,5 α , 25R)-3-[(4',6'-Bis[2-thienyl-methylcarbamoyl]-2',2',3',3',6'-penta-acetyl- β -D-cellobiosyl)oxy]-spirostan-11-one

Preparation D9

30 (3 β ,5 α , 25R)-3-[(4',6'-Bis[2-furan-2-yl-methylcarbamoyl]-2',2',3',3',6'-penta-acetyl- β -D-cellobiosyl)oxy]-spirostan-11-one

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Preparation D10

(3 β ,5 α , 25R)-3-[(4',6'-Bis[2-ethoxycarbonyl-propylcarbamoyl]-2',2',3',3',6'-penta-acetyl- β -D-cellobiosyl)oxy]-spirostan-11-one

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Preparation D11

(3 β ,5 α , 25R)-3-[(4',6'-Bis[ethoxycarbonyl-methylcarbamoyl]-2',2',3',3',6'-penta-acetyl- β -D-cellobiosyl)oxy]-spirostan-11-one

Preparation D12

10 (3 β ,5 α , 25R)-3-[(6'-[1-ethoxycarbonyl-2-(2-thienyl)-ethylcarbamoyl]-2',2',3',3',6'-penta-acetyl- β -D-cellobiosyl)oxy]-spirostan-11-one

Preparation D13

15 (3 β ,5 α , 25R)-3-[(6'-[1-ethoxycarbonyl-2-phenyl-ethylcarbamoyl]-2',2',3',3',6'-penta-acetyl- β -D-cellobiosyl)oxy]-spirostan-11-one

Preparation D14

20 (3 β ,5 α , 25R)-3-[(4',6'-Bis[1-ethoxycarbonyl-2-phenyl-ethyl)carbamoyl]-2',2',3',3',6'-penta-acetyl- β -D-cellobiosyl)oxy]-spirostan-11-one

Preparation D15

(3 β ,5 α , 25R)-3-[(4',6'-Bis[2-hydroxy-propylcarbamoyl]-2',2',3',3',6'-penta-acetyl- β -D-cellobiosyl)oxy]-spirostan-11-one

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Preparation D16

(3 β ,5 α , 12 β ,25R)-3-[(4',6'-Bis[ethoxycarbonyl-methylcarbamoyl]-2',2',3',3',6'-penta-acetyl- β -D-cellobiosyl)oxy]-12-acetoxy-spirostan-11-one

Preparation D17

30 (3 β ,5 α , 25R)-3-[(4',6'-Bis[2-methoxycarbonyl-ethylcarbamoyl]-2',2',3',3',6'-penta-acetyl- β -D-cellobiosyl)oxy]-spirostan-11-one

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Preparation D18

(3 β ,5 α , 25R)-3-[(4',6'-Bis[tetrahydro-furan-2-yl-methylcarbamoyl]-2',2',3',3',6'-penta-acetyl- β -D-cellobiosyl)oxy]-spirostan-11-one

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Preparation D19

(3 β ,5 α , 25R)-3-[(4',6'-Bis[3-thienyl-methylcarbamoyl]-2',2',3',3',6'-penta-acetyl- β -D-cellobiosyl)oxy]-spirostan-11-one

Preparation D20

10 (3 β ,5 α , 25R)-3-[(4',6'-Bis[2-(2-thienyl)-ethylcarbamoyl]-2',2',3',3',6'-penta-acetyl- β -D-cellobiosyl)oxy]-spirostan-11-one

Preparation D21

15 (3 β ,5 α , 25R)-3-[(4',6'-Bis[3,4-methylenedioxy-benzylcarbamoyl]-2',2',3',3',6'-penta-acetyl- β -D-cellobiosyl)oxy]-spirostan-11-one

Preparation D22

20 (3 β ,5 α , 25R)-3-[(4',6'-Bis[2-trifluoromethyl-benzylcarbamoyl]-2',2',3',3',6'-penta-acetyl- β -D-cellobiosyl)oxy]-spirostan-11-one

Preparation D23

(3 β ,5 α , 25R)-3-[(4',6'-Bis[5-methyl-2-thienyl-methylcarbamoyl]-2',2',3',3',6'-penta-acetyl- β -D-cellobiosyl)oxy]-spirostan-11-one

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Preparation D24

(3 β ,5 α , 25R)-3-[(4',6'-Bis[4-bromo-2-thienyl-methylcarbamoyl]-2',2',3',3',6'-penta-acetyl- β -D-cellobiosyl)oxy]-spirostan-11-one

Preparation D25

30 (3 β ,5 α , 25R)-3-[(4',6'-Bis[5-bromo-2-thienyl-methylcarbamoyl]-2',2',3',3',6'-penta-acetyl- β -D-cellobiosyl)oxy]-spirostan-11-one

Preparation D26

(3 β ,5 α , 12 β ,25R)-3-[(4',6'-Bis[3-thienyl-methylcarbamoyl]-2',2'',3',3'',6'-penta-acetyl- β -D-cellobiosyl)oxy]-12-hydroxy-spirostan-11-one

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Preparation D27

(3 β ,5 α , 25R)-3-[(4',6'-Bis[2-methoxycarbonyl-2-methyl-propylcarbamoyl]-2',2'',3',3'',6'-penta-acetyl- β -D-cellobiosyl)oxy]-spirostan-11-one

Preparation D28

10 (3 β ,5 α , 25R)-3-[(4',6'-Bis[2-hydroxy-2-(2-thienyl)-ethylcarbamoyl]-2',2'',3',3'',6'-penta-acetyl- β -D-cellobiosyl)oxy]-spirostan-11-one

Preparation D29

15 (3 β ,5 α ,12 β , 25R)-3-[(4',6'-Bis[2-methoxycarbonyl-ethylcarbamoyl]-2',2'',3',3'',6'-penta-acetyl- β -D-cellobiosyl)oxy]-12-acetoxy-spirostan-11-one

Preparation D30

(3 β ,5 α , 25R)-3-[(4',6'-Bis[thiazol-2-yl-methylcarbamoyl]-2',2'',3',3'',6'-penta-acetyl- β -D-cellobiosyl)oxy]-spirostan-11-one

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Preparation D31

(3 β ,5 α , 25R)-3-[(4',6'-Bis[benzylcarbamoyl]-2',2'',3',3'',6'-penta-acetyl- β -D-cellobiosyl)oxy]-spirostan-11-one

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Preparation D32

(3 β ,5 α , 25R)-3-[(4',6'-Bis[5-trifluoromethyl-benzothiazol-2-yl-methylcarbamoyl]-2',2'',3',3'',6'-penta-acetyl- β -D-cellobiosyl)oxy]-spirostan-11-one

Preparation D33

30 (3 β ,5 α , 25R)-3-[(4',6'-Bis[4-methoxy-benzylcarbamoyl]-2',2'',3',3'',6'-penta-acetyl- β -D-cellobiosyl)oxy]-spirostan-11-one

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Preparation D34

(3 β ,5 α , 25R)-3-[(4',6'-Bis[2-(2-fluorophenyl)ethylcarbamoyl]-2',2',3',3',6'-penta-acetyl- β -D-cellobiosyl)oxy]-spirostan-11-one

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Preparation D35

(3 β ,5 α , 12 β ,25R)-3-[(4',6'-Bis[thiazol-2-yl-methylcarbamoyl]-2',2',3',3',6'-penta-acetyl- β -D-cellobiosyl)oxy]-12-acetoxy-spirostan-11-one

Preparation D36

10 (3 β ,5 α , 25R)-3-[(4',6'-Bis[3-methylisoxazol-5-yl-methylcarbamoyl]-2',2',3',3',6'-penta-acetyl- β -D-cellobiosyl)oxy]-spirostan-11-one

Preparation D37

15 (3 β ,5 α , 25R)-3-[(4',6'-Bis[2-methoxycarbonyl-butylcarbamoyl]-2',2',3',3',6'-penta-acetyl- β -D-cellobiosyl)oxy]-spirostan-11-one

Preparation D38

20 (3 β ,5 α , 25R)-3-[(4',6'-Bis[pyridin-3-yl-methylcarbamoyl]-2',2',3',3',6'-pentaacetyl- β -D-cellobiosyl)oxy]-spirostan-11-one

Preparation E

(3 β ,5 α , 25R)-3-[(4',6'-Bis-[2-fluoro-phenylcarbamoyl]-2',2',3',3',6'-pentachloroacetyl- β -D-cellobiosyl)oxy]-spirostan-11-one

CARBAMOYLATION USING CUPROUS CHLORIDE

25 Cuprous chloride (1.74 g, 18 mmol) was added to a solution of (3 β ,5 α ,25R)-3-[(2',2',3',3',6'-penta-chloroacetyl- β -D-cellobiosyl)oxy]-spirostan-11-one (5.0 g, 4.4 mmol) and 2-fluorophenylisocyanate (1.98 mL, 18 mmol) in dry dimethyl formamide (30mL) at room temperature. After 2h, the mixture was diluted with ethyl acetate (100 mL) and washed with 1N HCl (2x) and brine (1x), dried (Na₂SO₄) filtered and
30 concentrated in vacuo. The residue was dissolved in methylene chloride (50 mL) and methanol (50 mL) was added. The methylene chloride was removed in vacuo and a solid precipitated from the methanol. The solid was filtered, washed with methanol and dried to afford 4.82 g product as a white solid (78%). m.p. 234-234.5

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°C. FAB MS: 1433 (M+Na)⁺. ¹H NMR (250 MHz, CDCl₃) δ 7.9 (m, 2H); 7.05 (m, 8H); 5.35 (dd, 1H, J = 8.0, 7.0 Hz); 5.28 (dd, 1H, J = 9.0, 8.0 Hz); 5.15 (dd, 1H, J = 9.0, 9.0 Hz); 5.05 (dd, 1H, J = 9.0, 8.0 Hz); 4.98 (dd, 1H, J = 8.0, 7.0 Hz); 4.72 (d, 1H, J = 9.0 Hz); 4.65-3.4 (m, 21 H); 3.35 (dd, 1H, J = 10.0, 9.0 Hz); 2.45 (m, 5 1H); 2.2 (s, 2H); 2.1-1.1 (m, 22 H); 1.0 (s, 3H); 0.9 (d, 3H, J = 7.0 Hz); 0.8 (d, 3H, J = 7.0 Hz); 0.7 (s, 3 H).

Preparation F

(3β,5α,25R)-3-[(6',6"-dideoxy-
pentaacetyl-β-D-cellobiosyl)oxy]spirostan-11-one

10

DEIODINATION

To a solution of (3β,5α,25R)-3-[(6',6"-dideoxy-6',6"-diiodo-pentaacetyl-β-D-cellobiosyl)oxy] spirostan-11-one (150 mg, 0.128 mmol) and tri-n-butyl tin hydride (0.105 mL, 0.39 mmol) in anhydrous toluene (5 mL), azoisobutyrylnitrile (10 mg) was added at room temperature. The reaction mixture was gently refluxed under
15 nitrogen atmosphere for 3 hours, cooled and concentrated in vacuo. The residual material was triturated with hexanes, filtered and dried to afford a colorless solid. The crude product was flash chromatographed (5% ethyl acetate/ methylene chloride) to afford 0.1 g (82%) of a white solid. ¹H NMR (250 MHz, CDCl₃) δ 5.1 (m, 2H); 4.8 (m, 2H); 4.5 (m, 2H); 4.1 (q, 1H, J=7Hz); 3.4 (m, 4H); 2.45 (bd, 1H,
20 J=14Hz); 2.25 (s, 2H); 2.12 (s, 3H); 2.10 (s, 3H); 2.09 (s, 3H); 2.06 (s, 3H); 1.98 (s, 3H); 1.9-1.3 (m, 25H); 1.3 (d, 3H, J=6Hz); 1.22 (d, 3H, J=6Hz); 1.0 (s, 3H); 0.9 (d, 3H, J=7Hz); 0.8 (d, 3H, J=7Hz); 0.7 (s, 3H).

Preparation G1

(3β,5α,25R)-3-[(6',6"-dideoxy-6',6"-difluoro-
pentaacetyl-β-D-cellobiosyl)oxy]spirostan-11-one

25

FLUORINATION

To a solution of (3β,5α,25R)-3-[(2',2",3',3",4"-pentaacetyl-β-D-cellobiosyl)oxy] spirostan-11-one (250 mg, 0.259 mmol) in anhydrous dimethoxy ether (5 mL), DAST (0.34 mL, 2.59 mmol) was added at 0°C. After 20 minutes, the reaction was warmed
30 to room temperature for 30 minutes, then to 40°C for 1.5 hours. Upon completion, the reaction was cooled to 0°C, diluted with ethyl acetate (50 mL) and poured into iced water. The organic layer was washed with 1N hydrochloric acid solution (1x), sodium bicarbonate solution (1x) and brine (1x), dried over sodium sulfate, filtered

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and concentrated in vacuo. The crude product was purified via flash chromatography (40% ethyl acetate/hexanes) to afford 190 mg (76 %) of a white solid. ¹HNMR (250 MHz, CDCl₃) δ 5.15 (dd, 1H, J=8,8Hz); 5.05 (dd, 1H, J=9,9 Hz); 4.9 (dd, 1H, J=9,8Hz); 4.85 (dd, 1H, J=9,8Hz); 4.7 (m, 1H); 4.5 (m, 4H); 3.85 (dd, 1H, J=9.0, 9.0Hz); 3.6 (m, 1H); 3.5 (m, 1H); 3.35 (dd, 1H, J=10.0, 11.0Hz); 2.5 (m, 1H); 2.2 (s, 2H); 2.01 (s, 3H); 2.0 (s, 6H); 1.98 (s, 3H); 1.97 (s, 3H); 1.9-1.0 (m, 27H); 1.0 (s, 3H); 0.92 (d, 3H, J=7Hz); 0.75 (d, 3H, J=7Hz); 0.7 (s, 3H). MS: 969 (M+H)⁺ m.p. 267-269°C.

In an analogous manner the following compounds, Preparations G2-G5, were prepared from the appropriate starting material using the above general procedure.

Preparation G2

(3 β ,5 α ,25R)-3-[(6'-deoxy-6'-fluoro-hexaacetyl- β -D-cellobiosyl)oxy]spirostan-11-one

15

Preparation G3

(3 β ,5 α ,25R)-3-[(6',6'-dideoxy-6',6'-difluoro-pentaacetyl- β -D-maltosyl)oxy]spirostan-11-one

20

Preparation G4

(3 β ,5 α ,12 β ,25R)-3-[(6',6'-dideoxy-6',6'-difluoro-pentaacetyl- β -D-cellobiosyl)oxy]-12-acetoxy-spirostan-11-one

25

Preparation G5

(3 β ,5 α ,12 β ,25R)-3-[(6'-deoxy-6'-fluoro-hexaacetyl- β -D-cellobiosyl)oxy]-12-acetoxy-spirostan-11-one

30

Preparation H1

(3 β ,5 α ,25R)-3-[(6',6'-dideoxy-6',6'-diiodo-pentaacetyl- β -D-cellobiosyl)oxy]spirostan-11-one

IODINATION

A mixture of (3 β ,5 α ,25R)-3-[(2',2',3',3',4'-pentaacetyl- β -D-cellobiosyl)oxy]spirostan-11-one (2.00g, 2.08 mmol), imidazole (0.85g, 12.0 mmol), and

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triphenylphosphine (3.26g, 12.0 mmol) was dissolved in toluene (40 mL). Iodine (2.10g, 8.30 mmol) was added and the reaction was gently refluxed overnight. Upon completion, the reaction was cooled, diluted with ethyl acetate, washed with 1N hydrochloric acid (1x), sodium bicarbonate solution (1x) and brine (1x), dried over sodium sulfate, filtered and concentrated in vacuo. The crude product was purified via flash chromatography (10% ethyl acetate/ methylene chloride) to afford 1.78g (72.3%) of a white solid.

¹HNMR (250 MHz, CDCl₃) δ 5.25-3.0 (m, 18H); 2.6-1.05 (m, 42H); 0.95 (d, 3H, J=7 Hz); 0.80 (s, 3H); 0.75 (d, 3H, J=7 Hz); 0.60 (s, 3H). MS: 1171 (M+H)⁺.

10

Preparation I1

(3β,5α,25R)-3-[(6',6"-dideoxy-6',6"-dichloro-pentaacetyl-β-D-cellobiosyl)oxy]spirostan-11-one

CHLORINATION

A mixture of (3β,5α,25R)-3-[(6',6"-dimethyl-pentaacetyl-β-D-cellobiosyl)oxy]spirostan-11-one (0.10g, 0.09 mmol), lithium chloride (0.15g) and N,N-dimethylformamide (2 mL) was heated to 85°C and stirred for 3 hours. The reaction was then cooled, diluted with ethyl acetate (20 mL), washed with water (2x) and brine (1x), dried over sodium sulfate, filtered and concentrated in vacuo. The residual material was triturated with hexanes, filtered and dried to afford 0.079g (88%) of a white solid.

20

¹HNMR (250 MHz, CDCl₃) δ 5.2-3.3 (m, 18H); 2.6-1.1 (m, 40H); 1.0 (s, 3H); 0.90 (d, 3H, J=7 Hz); 0.75 (d, 3H, J=7 Hz); 0.70 (s, 3H). MS: 1001 (M+H)⁺; m.p. >275°C.

In an analogous manner the following compound, Preparation I2, was prepared from the appropriate starting material using the above general procedure.

25

Preparation I2

(3β,5α,25R)-3-[(6',6"-dideoxy-6',6"-dichloro-pentaacetyl-β-D-lactosyl)oxy]spirostan-11-one

30

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Preparation J1

(3 β ,5 α ,25R)-3-[(6',6"-dimethyl-
pentaacetyl- β -D-cellobiosyl)oxy]spirostan-11-one

MESYLATION

5 A solution of (3 β ,5 α ,25R)-3-[(2',2",3',3",4"-pentaacetyl- β -D-cellobiosyl)oxy]spirostan-11-one (1.00g, 1.04 mmol) and triethylamine (1.50 mL, 10.40 mmol) in dichloromethane (10 mL) was cooled to 0°C. Mesityl chloride (0.48 mL, 6.22 mmol) and dimethylaminopyridine (0.02g) were added and the reaction was stirred at 0°C for 2 hours. The reaction was diluted with ethyl acetate (50 mL), washed with 1N
10 hydrochloric acid solution (2x), sodium bicarbonate solution (1x) and brine (1x), dried over sodium sulfate, filtered and concentrated in vacuo. The residual material was dissolved in dichloromethane (10 mL) and hexanes were added (20 mL). The dichloromethane was removed in vacuo and the resulting precipitate was filtered, washed with hexanes and dried under vacuum to afford 1.10g (94%) of a white
15 solid.

¹H NMR (250 MHz, CDCl₃) δ 5.25-3.3 (m, 18H); 3.1 (d, 6H, J=7 Hz); 2.6-1.1 (m, 40H); 1.0 (s, 3H); 0.95 (d, 3H, J=7 Hz); 0.77 (d, 3H, J=7 Hz); 0.7 (s, 3H).

In an analogous manner the following compound, Preparation J2,
was prepared from the appropriate starting material using the above general
20 procedure.

Preparation J2

(3 β ,5 α ,25R)-3-[(6',6"-dimesityl-
pentaacetyl- β -D-lactosyl)oxy]spirostan-11-one

25

Preparation K

(3 β ,5 α ,25R)-3-[(6',6"-Bis-(ethoxymethyl)-
pentaacetyl- β -D-cellobiosyl)oxy]spirostan-11-one

ETHOXYMETHYLATION

A mixture of (3 β ,5 α ,25R)-3-[(2',2",3',3",4"-pentaacetyl- β -D-cellobiosyl)oxy]spirostan-11-one (0.50 g, 0.52 mmol), dichloroethane (5 mL), diisopropylethylamine (1 mL) and ethoxymethyl chloride (0.196 g, 2.07 mmol) was stirred at room temperature for 4 hours. At this time, the reaction was warmed to 50°C for 2 hours. Upon completion, the reaction was cooled and quenched with methanol. The

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quenched mixture was diluted with ethyl acetate, washed with water (1x), 1N hydrochloric acid (2x) and brine (1x). The organic layer was then dried over sodium sulfate, filtered and concentrated in vacuo. The crude product was purified via flash chromatography (60% ethyl acetate/ 40% hexanes) to afford 0.5054 g

5 (46.7%). ¹H NMR (250 MHz, CDCl₃) δ 5.15 (dd, 1H, J=8.8Hz); 5.1 (m, 1H); 4.85 (m, 2H); 4.7 (s, 1H); 4.6 (s, 1H); 4.5 (dd, 1H, J=18.7Hz); 4.45 (m, 1H); 4.1 (q, 2H, J=6Hz); 3.8 (m, 2H); 3.5 (m, 10H); 2.5 (m, 1H); 2.2 (s, 2H); 2.01 (s, 3H); 2.0 (s, 6H); 1.98 (s, 3H); 1.97 (s, 3H); 1.9-1.0 (m, 26H); 1.2 (m, 6H); 1.0 (s, 3H); 0.92 (d, 3H, J=7Hz); 0.75 (d, 3H, J=7Hz); 0.7 (s, 3H).

10

Preparation L

(3β,5α,25R)-3-[(6'-deoxy-2',2'',3',3'',6'-penta-acetyl-β-D-cellobiosyl)oxy]-spirostan-11-one

2,2'-Azo-bis-(2-methyl)propionitrile (0.5 g) was added to a solution of tri-n-butyl tin hydride (3.73 mL, 13.9 mmol) and (3β,5α,25R)-3-[(6'-bromo-6'-deoxy-2',2'',3',3'',6'-pentaacetyl-β-D-cellobiosyl)oxy]-spirostan-11-one (9.54 g, 9.28 mmol) in 15 dry toluene (200 mL) at room temperature. The mixture was heated to a gentle reflux for 3h, was cooled and concentrated in vacuo. The product was purified by flash chromatography (70% ethyl acetate/hexanes) to afford 7.2 g of the title compound as a white solid (82%). m.p. 209-211 °C. FAB MS: 971 (M+Na)⁺. ¹H NMR (250 MHz, CDCl₃) δ 5.05 (dd, 1H, J = 10.0, 9.0 Hz); 4.85 (m, 3H); 4.48 (m, 4H); 4.05 (dd, 1H, J = 12.0, 5.0 Hz); 3.7 (dd, 1H, J = 9.0, 8.0 Hz); 3.45 (m, 6H); 2.45 (m, 1H); 2.2 (s, 2H); 2.1 (s, 3H); 2.05 (s, 3H); 2.0 (s, 9H); 2.0-1.1 (m, 23H); 1.0 (s, 3H); 0.95 (d, 3H, J = 7.0 Hz); 0.90 (d, 3H, J = 7.0 Hz); 0.8 (d, 3H, J = 7.0 Hz); 0.7 (s, 3H).

25

Preparation M

(3β,5α,25R)-3-[(6'-amino-6'-deoxy-2',2'',3',3'',6'-penta-acetyl-β-D-cellobiosyl)oxy]-spirostan-11-one . formate

AZIDE REDUCTION

10% Palladium on carbon (300 mg) was added to a solution of (3β,5α,25R)-3-[(6'-azido-6'-deoxy-2',2'',3',3'',6'-pentaacetyl-β-D-cellobiosyl)oxy]-spirostan-11-one (953 30 mg, 0.96 mmol) in methanol (15 mL) containing 2 mL of formic acid. The mixture was placed under 35 psi of hydrogen and shaken in a Parr apparatus for 2 h. The flask was purged with nitrogen and the catalyst was removed by filtration. The

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filtrate was concentrated in vacuo and the residue was purified by flash chromatography (2 to 8% methanol/ethyl acetate) to afford the amine as its formate salt as a white solid (600 mg, 62%). FAB MS: 986 (M+Na)⁺. ¹H NMR (250 MHz, CDCl₃) δ 5.15 (dd, 1H, J = 8.0, 8.0 Hz); 4.98 (dd, 1H, J = 7.0, 7.0 Hz); 4.8 (dd, 2H, J = 7.0, 7.0 Hz); 4.5 (m, 4H); 4.05 (dd, 1H, J = 12.0, 6.0 Hz); 3.7-3.0 (m, 12H); 2.5 (m, 1H); 2.25 (s, 2H); 2.12 (s, 3H); 2.08 (s, 3H); 2.02 (s, 3H); 2.0 (s, 6H); 2.0-1.0 (m, 22H); 1.0 (s, 3H); 0.9 (d, 3H, J = 7.0 Hz); 0.8 (d, 3H, J = 7.0 Hz); 0.7 (s, 3H).

Preparation N

10 (3β,5α,25R)-3-[(6"-azido-6"-deoxy-2',2',3',3',6'-pentaacetyl-β-D-cellobiosyl)oxy]-spirostan-11-one

BROMIDE DISPLACEMENT WITH SODIUM AZIDE

Sodium azide (1.98 g, 0.031 mol) was added to a solution of (3β,5α,25R)-3-[(6"-bromo-6"-deoxy-2',2',3',3',6'-pentaacetyl-β-D-cellobiosyl)oxy]-spirostan-11-one (7.84 g, 7.63 mmol) in dry DMF (60 mL) at room temperature. The mixture was heated to 60°C for 4.5 h, cooled, and diluted with ethyl acetate (100 mL). The mixture was washed with water (3x) and brine (1x), dried (Na₂SO₄), filtered and concentrated in vacuo. The product was triturated from ethyl acetate/hexanes, filtered and dried to afford 7.2 g of the title product as a white solid (95%). m.p. 164-166°C. FAB MS: 1012 (M+Na)⁺. ¹H NMR (250 MHz, CDCl₃) δ 5.15 (dd, 1H, J = 9.0, 8.0 Hz); 4.95 (dd, 1H, J = 13.0, 8.0 Hz); 4.8 (dd, 2H, J = 8.0, 7.0 Hz); 4.5 (m, 3H); 4.1 (dd, 1H, J = 12.0, 6.0 Hz); 3.75 (dd, 1H, J = 8.0, 7.0 Hz); 3.7-3.0 (m, 9H); 3.1 (d, 1H, J = 5.0 Hz); 2.4 (m, 1H); 2.2 (s, 2H); 2.1 (s, 3H); 2.08 (s, 6H); 2.02 (s, 3H); 2.0 (s, 3H); 2.0-1.0 (m, 23H); 1.0 (s, 3H); 0.9 (d, 3H, J = 7.0 Hz); 0.75 (d, 3H, J = 7.0 Hz); 0.7 (s, 3H).

Preparation O

(3β,5α,25R)-3-[(6"-bromo-6"-deoxy-2',2',3',3',6'-pentaacetyl-β-D-cellobiosyl)oxy]-spirostan-11-one

BROMINATION

30 Carbon tetrabromide (2.9 g, 8.7 mmol), triphenyl phosphine (5.4 g, 20.7 mmol) and pyridine (2.68 mL, 33 mmol) were added to a solution of (3β,5α,25R)-3-[(2',2',3',3',6'-pentaacetyl-β-D-cellobiosyl)oxy]-spirostan-11-one (8.0 g, 8.3 mmol) in methylene chloride (60 mL) at room temperature. After 19 h, the

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reaction was quenched by the addition of methanol (2 mL), diluted with methylene chloride (100 mL) and washed with NaHCO₃ sol. (1x), 1N HCl (1x) and brine (1x). The solution was dried (Na₂SO₄), filtered and concentrated in vacuo. The residue was purified by flash chromatography (50 % ethyl acetate/hexanes) to afford the

5 bromide as a white solid (7.9 g, 93 %). m.p. 218-220. FAB MS: 1050 (M+Na)⁺. ¹H NMR (250 MHz, CDCl₃) δ 5.15 (dd, 1H, J = 9.0, 8.0 Hz); 4.95 (dd, 1H, J = 13.0, 8.0 Hz); 4.8 (dd, 1H, J = 9.0, 2.0 Hz); 4.5 (m, 3H); 4.1 (dd, 1H, J = 12.0, 6.0 Hz); 3.85-3.4 (m, 10H); 3.3 (dd, 1H, J = 9.0, 9.0 Hz); 2.98 (d, 1H, J = 6.0 Hz); 2.45 (m, 1H); 2.2 (s, 2H); 2.1 (s, 6H); 2.08 (s, 3H); 2.02 (s, 6H); 2.0-1.0 (m, 22H);

10 1.0 (s, 3H); 0.9 (d, 3H, J = 7.0 Hz); 0.75 (d, 3H, J = 7.0 Hz); 0.7 (s, 3H).

Preparation P

(3 β ,5 α ,25R)-3-[(2',2'',3',3'',6'-penta-acetyl-6"-triisopropylsilyl- β -D-cellobiosyl)oxy]-

spirostan-11-one

MONOSILYLATION

15 Triisopropyl silyl chloride (0.37 mL, 1.7 mmol) was added to a solution of (3 β ,5 α ,25R)-3-[(2',2'',3',3'',6'-pentaacetyl- β -D-cellobiosyl)oxy]-spirostan-11-one (1.0g, 1.04 mmol) in dry dimethyl formamide (5 mL) containing imidazole (141 mg, 2.07 mmol) and N,N-dimethyl 4-amino pyridine (50 mg). After 24 h, an additional 0.1 mL of triisopropyl silyl chloride was added and the mixture stirred another 24 h. The

20 mixture was diluted with ethyl acetate (50 mL) and washed with water (3x), 1N HCl (2x) and brine (1x), dried (Na₂SO₄), filtered and concentrated in vacuo. The residue was purified by flash chromatography (40 % ethyl acetate/hexanes) to afford the title compound as a colorless foam (690 mg, 60 %). ¹H NMR (250 MHz, CDCl₃) δ 5.12 (dd, 1H, J = 9.0, 8.0 Hz); 5.05 (dd, 1H, J = 9.0, 8.0 Hz); 4.8 (ddd, 1H, J = 8.0,

25 8.0, 2.0 Hz); 4.5 (m, 2H); 4.1 (m, 1H); 3.9-3.3 (m, 13H); 2.5 (m, 1H); 2.25 (s, 2H); 2.1 (s, 3H); 2.08 (s, 3H); 2.04 (s, 6H); 2.02 (s, 3H); 1.9-1.05 (m, 43H); 1.0 (s, 3H); 0.9 (d, 3H, J = 7.0 Hz); 0.78 (d, 3H, J = 7.0 Hz); 0.7 (s, 3H).

Preparation Q

(3 β ,5 α ,25R)-3-[(2',2'',3',3'',6'-penta-chloroacetyl

- β -D-cellobiosyl)oxy]-spirostan-11-one

PARAMETHOXY BENZYLIDENE HYDROLYSIS

30 Trifluoroacetic acid (19 mL) was added to a solution of (3 β ,5 α ,25R)-3-[(4',6'-[4-methoxybenzyliden]-2',2'',3',3'',6'-penta-chloro-acetyl- β -D-cellobiosyl)oxy]-

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spir stan-11-one (23.7 g, 0.019 mol) in dichloromethane (150 mL) and methanol (50 mL). After 4 h, the mixture was washed with water (3x) NaHCO₃ (2x) and brine (1x), dried (Na₂SO₄) filtered and concentrated. The residue was dissolved in a minimal amount of ethyl acetate and precipitated with hexanes. The solid was
 5 filtered and washed with hexanes and dried to afford 19.7 g product as a white solid (92 %). m.p. 228-229. FAB MS: 1159 (M+Na)⁺. ¹H NMR (250 MHz, CDCl₃) δ 5.2 (dd, 1H, J = 9.0, 9.0 Hz); 5.1 (dd, 1H, J = 9.0, 9.0 Hz); 4.95 (m, 2H); 4.6 (m, 3H); 4.5 (ddd, 1H, J = 8.0, 7.0, 7.0 Hz); 4.2-3.4 (m, 20H); 3.35 (dd, 1H, J = 9.0, 9.0 Hz); 2.9 (d, 1H, J = 6.0 Hz); 2.45 (m, 1H); 2.2 (s, 2H); 2.1-1.1 (m, 22H); 1.0 (s,
 10 3H); 0.94 (d, 3H, J = 7.0 Hz); 0.77 (d, 3H, J = 7.0 Hz); 0.7 (s, 3H).

Preparation R

(3 β ,5 α ,25R)-3-[(4',6'-[4-methoxybenzylidene]-2',2',3',3',6'-penta-chloro-acetyl)- β -D-cellobiosyl)oxy]-spirostan-11-one

PARAMETHOXYBENZYLIDENE FORMATION AND CHLOROACETYLTATION

15 Camphorsulfonic acid (3g) was added to a mixture of (3 β ,5 α ,25R)-3-[(β -D-cellobiosyl)oxy]-spirostan-11-one (50 g, 0.066 mol) and anisaldehyde dimethyl acetal (50 mL, 0.29 mol) in 1,2-dichloroethane (1500 mL). The suspension was heated to reflux temperature and 200 mL of solvent was distilled off. After 4h at reflux temperature, the dark, gelatinous mixture was cooled to 0°C and treated with
 20 pyridine (160 mL, 1.99 mol) and chloroacetic anhydride (170 g, 1 mmol). The reaction was allowed to warm to room temperature and after 2 h, the mixture was washed with 1N HCl (3x), NaHCO₃ (1x) and brine (1x), dried (Na₂SO₄), filtered and concentrated in vacuo. The residue was dissolved in a minimum amount of ethyl acetate and the product was precipitated with hexanes. The solid was filtered,
 25 washed with hexanes and dried to afford 77 g product as a white solid (93%). m.p. 256-257. FAB MS: 1277 (M+Na)⁺. ¹H NMR (250 MHz, CDCl₃) δ 7.35 (d, 2H, J = 9.0 Hz); 6.88 (d, 2H, J = 9.0 Hz); 5.45 (s, 1H); 5.3 (m, 2H); 5.0 (m, 2H); 4.7 (d, 1H, J = 7.0 Hz); 4.6 (m, 2H); 4.5 (dd, 1H, J = 8.0, 8.0 Hz); 4.38 (dd, 1H, J = 11.0, 6.0 Hz); 4.2-3.5 (m, 15H); 3.8 (s, 3H); 3.4 (dd, 1H, J = 11.0, 10.0 Hz); 2.5
 30 (m, 1H); 2.25 (s, 2H); 2.1-1.0 (m, 25H); 1.0 (s, 3H); 0.94 (d, 3H, J = 7.0 Hz); 0.78 (d, 3H, J = 7.0 Hz); 0.7 (s, 3H).

Preparation S1

(3 β ,5 α ,25R)-3-[(2',2'',3',3',6'-
pentaacetyl- β -D-cellobiosyl)oxy]spirostan-11-one

BENZYLIDENE HYDROLYSIS

5 (3 β ,5 α ,25R)-3-[(4''6''-benzylidene-pentaacetyl- β -D-cellobiosyl)-oxy]spirostan-11-one (6.5g,6.16mmol) was added to glacial acetic acid (80 mL) and water (20 mL). The reaction was heated to 80°C and stirred for 2 hours under nitrogen atmosphere. Upon completion, the reaction was cooled and then was added to ice. The organic material was extracted with ethyl acetate, then washed with water (1x),
 10 saturated sodium bicarbonate solution (1x), water (1x) and brine (1x). The organic layer was dried over sodium sulfate, filtered and concentrated in vacuo. The residual material was triturated with hexanes, filtered, washed with hexanes and dried to afford 4.4 g (75% yield) of product.

¹HNMR (250MHz, CDCl₃) δ 5.25-3.3 (m, 19H); 2.95 (d, 1H, J=7 Hz); 2.6-1.1 (m, 40H); 1.0 (s, 3H); 0.90 (d, 3H, J=7 Hz); 0.8 (d, 3H, J=7 Hz); 0.7 (s, 3H).
 15 MS: 965 (M+H)⁺ m.p. 210-212°C

In an analogous manner the following compounds, Preparation S2-S3, were prepared from the appropriate starting material using the above general procedure.

Preparation S2

20 (3 β ,5 α ,12 β ,25R)-3-[(2',2'',3',3',6'-
pentaacetyl- β -D-cellobiosyl)oxy]12-acetoxyspirostan-11-one

Preparation S3

(3 β ,5 α ,11 β ,25R)-3-[(2',2'',3',3',6'-pentaacetyl-
 25 β -D-cellobiosyl)oxy]-11-acetoxy-spirostane

Preparation T1

(3 β ,5 α ,25R)-3-[(4'',6''-benzylidene-
pentaacetyl- β -D-cellobiosyl)oxy]spirostan-11-one

BENZYLIDENE FORMATION AND ACETYLATION

30 A mixture of (3 β ,5 α ,25R)-3-[(β -D-cellobiosyl)]oxy]spirostan-11-one(5.00 g, 6.63 mmol), camphorsulphonic acid (0.75 g), chloroform (200 mL) and benzaldehyd dimethyl acetal (5.00 mL) was h ated to reflux temperature under

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nitrogen atmosphere. The reaction was stirred at reflux temperature for 5 hours. Upon formation of the benzylidene, the reaction was cooled in an ice bath and pyridine (16 mL, 0.19 mol), dimethylaminopyridine (2.00 g) and acetic anhydride (10 mL, 0.11 mol) were added. The reaction was warmed to room temperature and

5 stirred overnight. The reaction mixture was then washed with water once. The reaction was partially concentrated in vacuo to remove most of the chloroform. The remaining material was diluted with ethyl acetate, washed with 1N hydrochloric acid solution (3x), water (1x) and brine (1x). The organic layer was then dried over sodium sulfate, filtered and concentrated in vacuo. The residual material was

10 triturated with hexanes, filtered, washed with hexanes and dried to afford 6.5 g (93% yield) of product. ¹HNMR (250 MHz, CDCl₃) δ 7.45-7.3 (m, 5H); 5.5 (s, 1H); 5.3-3.3 (m, 18H); 2.5-1.05 (m, 40H); 1.0 (s, 3H); 0.90 (d, 3H, J=7 Hz); 0.8 (d, 3H, J=7 Hz); 0.7 (s, 3H).

in an analogous manner the following compounds, Preparation T2-T3, were

15 prepared from the appropriate starting material using the above general procedure.

Preparation T2

(3 β ,5 α ,12 β ,25R)-3-[(4',6'-benzylidene-pentaacetyl- β -D-cellobiosyl)oxy]-12-acetoxy-spirostan-11-one

20

Preparation T3

(3 β ,5 α ,11 β ,25R)-3-[(4',6'-benzylidene-pentaacetyl- β -D-cellobiosyl)oxy]-11-acetoxy-spirostan-11-one

Preparation U1

25

(3 β ,5 α ,25R)-3-[(2',2',3',3',4'-pentaacetyl- β -D-cellobiosyl)oxy]spirostan-11-one

DESILYLATION

A mixture of (3 β ,5 α ,25R)-3-[(6',6'-bis-[t-butylidiphenylsilyl]-pentaacetyl- β -D-cellobiosyl)oxy] spirostan-11-one (15.04 g, 0.01 mol) and pyridine (100 mL) was

30 cooled to 0°C under nitrogen atmosphere. Hydrogen fluoride in pyridine (36.64 mL) was added and the reaction was gradually warmed to room temperature and allowed to stir for 4 hours. Upon completion, the reaction was cooled and quenched with water. The quenched mixture was dissolved in ethyl acetate, washed

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with water (1x), 1N hydrochloric acid (4x), brine (1x), dried over sodium sulfate, filtered and concentrated in vacuo. The residual material was triturated with hexanes, filtered, washed with hexanes and dried to afford 7.22g (74.8%) of product.

^1H NMR (250 MHz, CDCl_3) δ 5.25-3.3 (m, 20H); 2.65-1.05 (m, 40H); 1.0 (s, 3H); 0.95 (d, 3H, $J=8$ Hz); 0.8 (d, 3H, $J=8$ Hz); 0.7 (s, 3H).
MS: 965 (M+H) $^+$; m.p. 233-234°C.

In an analogous manner the following compounds, Preparations U2-U4, were prepared from the appropriate starting material using the above general procedure.

10

Preparation U2

(3 β ,5 α ,25R)-3-[(2',2'',3',3'',4''-pentaacetyl- β -D-lactosyl)oxy]spirostan-11-one

15

Preparation U3

(3 β ,5 α ,25R)-3-[(2',2'',3',3'',4''-pentaacetyl- β -D-maltosyl)oxy]spirostan-11-one

20

Preparation U4

(3 β ,5 α ,12 β ,25R)-3-[(2',2'',3',3'',4''-pentaacetyl- β -D-cellobiosyl)oxy]-12-acetoxy-spirostan-11-one

25

Preparation V1

(3 β ,5 α ,25R)-3-[(6',6''-di-(*t*-butyldiphenylsilyl)-pentaacetyl- β -D-cellobiosyl)oxy]spirostan-11-one

SILYLATION AND ACETYLATION

A mixture of (3 β ,5 α ,25R)-3-[(β -D-cellobiosyl)oxy]spirostan-11-one (25 g, 0.03 mol), imidazole (15.78 g, 0.23 mol), dimethylaminopyridine (20 g) and *N,N*-dimethylformamide (400 mL) was cooled to 0°C under nitrogen atmosphere. Tert-butyldiphenylsilyl chloride (34.45 mL, 0.13 mol) was added, the mixture was warmed to room temperature and stirred for 5 hours. Pyridine (53.57 mL, 0.66 mol) and acetic anhydride (54.87 mL, 0.50 mol) were added and the reaction was stirred overnight. The reaction was then quenched with water, diluted with ethyl acetate, washed with water (2x), 1N hydrochloric acid solution (3x), brine (1x), dried over

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sodium sulfate, filtered and concentrated in vacuo to afford a foam as the product (66.05 g, >100%). ¹H NMR (250 MHz, CDCl₃) δ 7.85-7.3 (m, 20H); 5.3-3.3 (m, 18H); 2.6-1.0 (m, 61H); 0.90 (d, 3H, J=7 Hz); 0.8 (d, 3H, J=7 Hz); 0.7 (s, 3H). m.p. 135°C.

5 In an analogous manner the following compounds, Preparations V2-V4, were prepared from the appropriate starting material using the above general procedure.

Preparation V2

10 (3β,5α,25R)-3-[(6',6"-di-(t-butylidiphenylsilyl)-pentaacetyl-β-D-lactosyl)oxy]spirostan-11-one

Preparation V3

15 (3β,5α,25R)-3-[(6',6"-di-(t-butylidiphenylsilyl)-pentaacetyl-β-D-maltosyl)oxy]spirostan-11-one

Preparation V4

(3β,5α,12β,25R)-3-[(6',6"-di-(t-butylidiphenylsilyl)-pentaacetyl-β-D-cellobiosyl)oxy]-12-acetoxy-spirostan-11-one

20 Preparation W1

(3β,5α,25R)-3-[(β-D-cellobiosyl)oxy]spirostan-11-one

DEACETYLATION

A mixture of (3β,5α,25R)-3-[(heptaacetyl-β-D-cellobiosyl)oxy]spirostan-11-one (6.57 g, 6.26 mmol), sodium methoxide (68 mg, 1.25 mmol), methanol (35 mL) and
 25 tetrahydrofuran (75 mL) was heated to reflux for 1 hour, followed by stirring at room temperature for 12 hours. A white precipitate formed within 30 minutes. The final suspension was concentrated in vacuo to give 6.0 g of crude product. This material was purified by flash chromatography (eluent: chloroform followed by 8:2 chloroform:methanol) to give 2.71 g (57% yield) of the title compound. High
 30 resolution FAB MS (m/e): calculated for C₃₉H₆₂O₁₄Na 777.4037, found 777.4108. Analysis: calc. for C₃₉H₆₂O₁₄-2H₂O, C 59.22 H 8.41; found C 59.48, H 8.48. MP: >300°C.

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In an analogous manner the following compounds W2-W5 were prepared from the appropriate starting material using the above general procedure.

Preparation W2

(3 β ,5 α ,25R)-3-[(β -D-lactosyl)oxy]-spirostan-11-one

5

Preparation W3

(3 β ,5 α ,25R)-3-[(β -D-maltosyl)oxy]-spirostan-11-one

Preparation W4

10 (3 β ,5 α ,12 β ,25R)-3-(β -D-cellobiosyl)oxy]-12-hydroxy-spirostan-11-one

Preparation W5

(3 β ,5 α ,11 β ,25R)-3-(β -D-cellobiosyl)oxy]-11-hydroxy-spirostane

15

Preparation X

(3 β ,5 α ,25R)-3-[(Heptaacetyl- α -D-cellobiosyl)oxy]spirostan-11-one

ANOMERIZATION

Hydrobromic acid (30% in acetic acid, 1.2 mL) was added to a room temperature solution of (3 β ,5 α ,25R)-3-[(heptaacetyl- β -D-cellobiosyl)oxy]spirostan-
20 11-one (2.0 g) in methylene chloride (35 mL) and the resulting mixture was stirred at room temperature for 94 hours. The reaction was quenched by slow addition of saturated aqueous sodium bicarbonate (20 mL). The organic layer was separated, dried over magnesium sulfate, and dried in vacuo to give 1.637 g of a black solid. Purification by repeated flash chromatography (1:1 hexane:ethyl acetate eluent)
25 provided 651 mg (33% yield) of the title compound.

MS (m/e): 1049 (M+H), 1071 (M + Na). Analysis: calc. for C₅₃H₇₆O₂₁·H₂O, C 59.65 H 7.37; found C 59.66 H 7.00. MP: 248-249°C.

Preparation Y1

(3 β ,5 α ,25R)-3-[(Heptaacetyl- β -D-cellobiosyl)oxy]spirostane-11-one

30

ZINC FLUORIDE PROMOTED COUPLING OF FREE SPIROSTANE

A suspension of (3 β ,5 α ,25R)-3-hydroxyspirostan-11-one (3.0 g, 6.97 mmol) and anhydrous zinc fluoride (2.88 g, 27.9 mmol) in dry acetonitrile (175 mL) was dried by removal of 75 mL of acetonitrile by distillation. The suspension was

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allowed to cool, heptaacetyl- β -D-cellobiosyl bromide (9.75 g, 13.9 mmol) was added and the resulting suspension was heated to 65°C for 3 hours. After cooling to room temperature, methylene chloride (150 mL) was added, the suspension was stirred for 10 minutes and filtered. The filtrate was concentrated in vacuo to give 10 g of
 5 crude product. This material was dissolved in 8:2 chloroform:methanol, preadsorbed on silica gel and purified by flash chromatography (eluent: 1:1 ethyl acetate:hexane followed by pure ethyl acetate) to give 6.81 g (93% yield) of the title material.

MS (m/e): 1049 (M+H). Analysis: calc. for $C_{53}H_{76}O_{21} \cdot H_2O$, C 59.65, H 7.37;
 10 found C 59.86, H 7.25. MP: 210-212°C.

In an analogous manner the following compounds Y2-Y4 were prepared from the appropriate starting material using the above general procedure.

Preparation Y2

(3 β ,5 α ,25R)-3-[(Hepta-acetyl- β -D-lactosyl)oxy]-spirostan-11-one
 15

Preparation Y3

(3 β ,5 α ,12 β ,25R)-3-[(Hepta-acetyl- β -D-cellobiosyl)oxy]-12-acetoxy-spirostan-11-one

Preparation Y4

20 (3 β ,5 α ,11 β ,25R)-3-[(Hepta-acetyl- β -D-cellobiosyl)oxy]-11-acetoxy-spirostan-11-one

Preparation Z

(3 β ,5 α ,25R)-3-[(heptaacetyl- β -D-maltosyl)oxy]-spirostan-11-one

MERCURIC BROMIDE/MERCURIC CYANIDE

25 PROMOTED COUPLING OF Silylated SPIROSTANE

Powdered 4A molecular sieves (4g) were added to a solution of 3-trimethylsilyloxy-(3 β ,5 α ,25R)spirostan-11-one (3.90 g, 7.76 mmol) and acetobromomaltose (8.15 g, 11.7 mmol) in dichloromethane (60 mL) at room temperature. After stirring for 15 minutes $Hg(CN)_2$ (7.85 g, 31 mmol) and $HgBr_2$
 30 (11.2 g, 31 mmol) were added and the mixture stirred at room temperature for 7 hours. The mixture was diluted with ethyl acetate and washed with 1N hydrochloric acid (3x) and brine (1x), dried over sodium sulfate, filtered and concentrated in vacuo. The product was purified by flash chromatography (30-60% EtOAc/hexanes)

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and afforded 3.5g (43%) of the title product. ¹H NMR (250 MHz, CDCl₃) δ 5.4 (d, 1H, J=3Hz); 5.3 (dd, 1H, J=9.9Hz); 5.2 (dd, 1H, J=8.8Hz); 5.05 (dd, 1H, J=9.9Hz); 4.88 (dd, 1H, J=11.3Hz); 4.7 (dd, 1H, J=10.8Hz); 4.4 (m, 2H); 4.2 (m, 2H); 4.0 (m, 3H); 3.5 (m, 4H); 2.5 (m, 1H); 2.2 (s, 2H); 2.16 (s, 3H); 2.1 (s, 3H); 2.02 (s, 3H); 2.0 (s, 3H); 1.98 (s, 3H); 1.96 (s, 3H); 1.95 (s, 3H); 1.95-1.0 (m, 23H); 1.0 (s, 3H); 0.9 (d, 3H, J=7Hz); 0.65 (d, 3H, J=7Hz); 0.55 (s, 3H).

Preparation AA

(3β,5α,25R)-3-trimethylsilyloxy-spirostan-11-one

SILYLATION OF SPIROSTANES

10 Trimethylsilylchloride (3.27 mL, 25.8 mmol) was added to a solution of (3β,5α,25R)-3-hydroxy-spirostan-11-one (4.0 g, 9.3 mmol) and triethylamine (6.5 mL, 46 mmol) in dichloromethane (60 mL) at room temperature. One gram of dimethyl aminopyridine was added and the reaction was stirred at room temperature for 12 hours. The reaction was quenched with methanol (1 mL) and diluted with ethyl
15 acetate, washed with water (5x) and brine (1x), dried (Na₂SO₄), filtered and concentrated in vacuo. The product was triturated with methanol, filtered and dried to afford 3.94 g (85%) product as a white solid. ¹H NMR (250 MHz, CDCl₃) δ 4.5 (q, 1H, J=6Hz); 3.45 (m, 2H); 2.35 (t, 1H, J=10Hz); 2.4 (dt, 1H, J=12.2Hz); 2.2 (s, 2H); 2.1-1.1 (m, 12H); 1.02 (s, 3H); 0.9 (d, 3H, J=7.0Hz); 0.78 (d, 3H, J=7Hz); 0.69 (s, 20 3H); 0.1 (s, 9H).

Preparation BB1

(3β,5α,11β,12α,25R)-spirostan-3,11,12-triol

(3β,5α,11α,25R)-11,23-dibromo-3-acetoxyspirostan-12-one: The title compound was synthesized from (3β,5α,25R)-3-acetoxyspirostan-12-one according
25 to the procedure described in J. Chem. Soc., 1956, 4344.

(3β,5α,11α,12β,25R)-11,23-dibromospirostan-3,12-diol: (3β,5α,11α,25R)-11,23-dibromo-3-acetoxyspirostan-12-one (20.00 g, azeotropically dried with toluene) was dissolved in THF (600 mL) and cooled to -78°C. Lithium aluminum hydride (96.0 mL of 1.0 M THF solution) was slowly added and the resulting mixture was
30 stirred at -78°C for 2 hours and 0°C for 0.5 hour. Using a cannula, the mixture was cautiously transferred into stirred 3 M aqueous ammonium chloride (200 mL). The organic phase was separated, combined with THF washes of the solid residues, and concentrated to give the title compound.

- (3 β ,5 α ,11 β ,12 β ,25R)-23-bromo-11,12-epoxyspirostan-3-ol: The following procedure is a variation of that described in Helv. Chim. Act., 1953, 36, 1241. (3 β ,5 α ,11 α ,12 β ,25R)-11,23-dibromospirostan-3,12-diol (18.08 g) was dissolved in pyridine (500 mL) at room temperature and treated with silver oxide (70.0 g). The
- 5 resulting mixture was stirred in the dark for 71 hours. The mixture was filtered and the solid washed with ether and then chloroform. These washes were combined with the filtrate and concentrated. The resulting solid was purified by flash chromatography (1:1 hexane:ethyl acetate) to give 12.2 g of a 1:1 mixture of the title compound and (3 β ,5 α ,25R)-23-bromospirostan-3-ol-12-one. Further
- 10 chromatography (7:3 hexane:ethyl acetate) provides pure title compound.

(3 β ,5 α ,11 β ,12 α ,25R)-23-bromo-12-(trichloroacetoxyspirostan-3,11-diol: Using the procedure described in J. Chem. Soc., 1956, 4330, (3 β ,5 α ,11 β ,12 β ,25R)-23-bromo-11,12-epoxyspirostan-3-ol was treated with trichloroacetic acid in toluene at room temperature for 3 days to give the title compound.

- 15 (3 β ,5 α ,11 β ,12 α ,25R)-23-bromo-spirostan-3,11,12-triol: Using the procedure described in J. Chem. Soc., 1956, 4330, (3 β ,5 α ,11 β ,12 α ,25R)-23-bromo-12-(trichloroacetoxyspirostan-3,11-diol was saponified with sodium hydroxide in water and ethanol to give the title compound.

- (3 β ,5 α ,11 β ,12 α ,25R)-spirostan-3,11,12-triol: Using the procedure described in
- 20 J. Chem. Soc., 1956, 4330, (3 β ,5 α ,11 β ,12 α ,25R)-23-bromo-12-(trichloroacetoxyspirostan-3,11-diol was reduced with zinc and acetic acid to give the title compound.

Preparation BB2

(3 β ,5 α ,12 α ,25R)-spirostan-3,12-diol-11-one

- (3 β ,5 α ,11 β ,12 α ,25R)-3,12-di(acetoxyspirostan-11-ol: Using the procedure
- 25 described in J. Chem. Soc., 1956, 4330, (3 β ,5 α ,11 β ,12 α ,25R)-spirostan-3,11,12-triol(preparation G1) was selectively acetylated with acetic anhydride and pyridine to give the title compound.

- (3 β ,5 α ,12 α ,25R)-3,12-di(acetoxyspirostan-11-one: Using the procedure described in Org. Syn., 1976, 55, 84, (3 β ,5 α ,11 β ,12 α ,25R)-3,12-di(acetoxyspirostan-
- 30 11-ol was oxidized with chromium trioxide and pyridine in methylene chloride to give the title compound.

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(3 β ,5 α ,12 α ,25R)-spirostan-3,12-diol-11-one: Using the procedure described in Syn., 1973, 790, (3 β ,5 α ,12 α ,25R)-3,12-di(acetoxy)spirostan-11-one was saponified with potassium cyanide in water, methanol and THF to give the title compound.

Preparation BB3

5

(3 β ,5 α ,11 β ,25R)-spirostan-3,11-diol

(3 β ,5 α ,25R)-spirostan-3-ol-11-one (Aldrich Chemical Company, Milwaukee, WI or Steraloids Inc., Wilton, N.H., or see preparation G13) was converted into the title compound via reduction with lithium aluminum hydride in THF at room temperature according to the procedure described in J. Am. Chem. Soc., 1951, 73, 1777.

10

Preparation BB4

(3 β ,5 α ,11 α ,25R)-spirostan-3,11-diol

(3 β ,5 α ,25R)-spirostan-3-ol-11-one (Aldrich Chemical Company, Milwaukee, WI or Steraloids Inc., Wilton, N.H., or see preparation G13) was converted into the title compound via reduction with lithium and ammonia according to the procedure described in J. Am. Chem. Soc., 1953, 75, 1282.

Preparation BB5

(3 β ,5 α ,11 β ,12 β ,25R)-spirostan-3,11,12-triol

(3 β ,5 α ,12 β ,25R)-3,12-di(acetoxy)spirostan-11-one (purchased from Steraloids, Inc., or see preparation G13) was converted into the title compound via reduction with lithium aluminum hydride in THF at room temperature according to the procedure described in J. Am. Chem. Soc., 1951, 73, 1777.

Preparation BB6

(3 β ,5 α ,11 α ,12 β ,25R)-spirostan-3,11,12-triol

(3 β ,5 α ,12 β ,25R)-spirostan-3,12-diol-11-one: (3 β ,5 α ,12 β ,25R)-3,12-di(acetoxy)-spirostan-11-one (purchased from Steraloids, Inc., or see preparation G13) was saponified with potassium carbonate in water, methanol and THF to provide the title compound.

(3 β ,5 α ,11 α ,12 β ,25R)-spirostan-3,11,12-triol: (3 β ,5 α ,12 β ,25R)-spirostan-3,12-diol-11-one was converted into the title compound via reduction with lithium and ammonia according to the procedure described in J. Am. Chem. Soc., 1953, 75, 1282.

30

Preparation BB7(3 β ,5 α ,25R-)spirostan-3-ol-11,12-dione

(3 β ,5 α ,12 β ,25R)-3-(t-butyldimethylsilyloxy)spirostan-12-ol-11-one: Using the procedure described in J. Am. Chem. Soc., 1972, 94, 6190, (3 β ,5 α ,12 β ,25R)-

- 5 spirostan-3,12-diol-11-one (see preparation G6) was silylated with t-butyldimethylchlorosilane and imidazole in DMF to give the title compound.

- (3 β ,5 α ,25R)-3-(t-butyldimethylsilyloxy)spirostan-11,12-dione: Using the procedure described in Org. Syn., 1976, 55, 84, (3 β ,5 α ,12 β ,25R)-3-(t-butyldimethylsilyloxy)spirostan-12-ol-11-one was oxidized with chromium trioxide
10 and pyridine in methylene chloride to give the title compound.

(3 β ,5 α ,25R)-spirostan-3-ol-11,12-dione: Using the procedure described in J. Am. Chem. Soc., 1972, 94, 6190, (3 β ,5 α ,25R)-3-(t-butyldimethylsilyloxy)spirostan-11,12-dione was desilylated with hydrofluoric acid in acetonitrile to give the title compound.

15

Preparation BB8(3 β ,5 α ,11 β ,25R-)spirostan-3,11-diol-12-one(3 β ,5 α ,11 β ,12 β ,25R-)3-(t-butyldimethylsilyloxy)spirostan-11,12-diol:

- (3 β ,5 α ,12 β ,25R)-3-(t-butyldimethylsilyloxy)spirostan-12-ol-11-one (see procedure G8) was converted into the title compound via reduction with lithium aluminum hydride in
20 THF at room temperature according to the procedure described in J. Am. Chem. Soc., 1951, 73, 1777.

(3 β ,5 α ,11 β ,12 β ,25R-)3-(t-butyldimethylsilyloxy)-12-acetoxyspirostan-11-ol:

- (3 β ,5 α ,11 β ,12 β ,25R)-3-(t-butyldimethylsilyloxy)spirostan-11,12-diol was selectively acetylated with acetic anhydride, pyridine and dimethylaminopyridine in methylene
25 chloride to give the title compound.

(3 β ,5 α ,11 β ,12 β ,25R-)3-(t-butyldimethylsilyloxy)-11-(trimethylsilyloxy)-12-acetoxyspirostane: (3 β ,5 α ,11 β ,12 β ,25R)-3-(t-butyldimethylsilyloxy)-12-

- acetoxyspirostan-11-ol was silylated with trimethylsilyltriflate and 2,6-lutidine in methylene chloride according to the procedure described in Tetrahedron Letters,
30 1981, 22, 3455.

(3 β ,5 α ,11 β ,12 β ,25R-)3-(t-butyldimethylsilyloxy)-11-(trimethylsilyloxy)spirostan-12-ol: (3 β ,5 α ,11 β ,12 β ,25R)-3-(t-butyldimethylsilyloxy)-11-(trimethylsilyloxy)-12-

acet xyspirostane was d acetylated by treatment with lithium aluminum hydrid in

THF followed by cautious addition aqueous ammonium chloride. The resulting title compound suffered 11 to 12 silyl group migration on silica gel, and thus had to be used unpurified.

- 5 (3 β ,5 α ,11 β ,25R)-3-(t-butyldimethylsilyloxy)-11-(trimethylsilyloxy)spirostan-12-one: (3 β ,5 α ,11 β ,25R)-3-(t-butyldimethylsilyloxy)-11-(trimethylsilyloxy)spirostan-12-ol was oxidized with chromium trioxide and pyridine in methylene chloride according to the procedure described in Org. Syn., 1976, 55, 84 to give the title compound.

- 10 (3 β ,5 α ,11 β ,25R)-spirostan-3,11-diol-12-one: (3 β ,5 α ,11 β ,25R)-3-(t-butyldimethylsilyloxy)-11-(trimethylsilyloxy)spirostan-12-one was desilylated with hydrofluoric acid in acetonitrile according to the procedure described in J. Am. Chem. Soc., 1972, 94, 6190 to give the title compound. The title compound must be carefully handled because it will rearrange to (3 β ,5 α ,12 β ,25R)-spirostan-3,12-diol-11-one if exposed to base.

15 Preparation BB9

(3 β ,5 α ,11 α ,25R)-spirostan-3,11-diol-12-one

- (3 β ,5 α ,11 α ,25R)-3,11-di(acetoxy)spirostan-12-ol: (3 β ,5 α ,11 α ,25R)-spirostan-3,11,12-triol (see preparation G6) was acetylated according to the procedure described in J. Am. Chem. Soc., 1955, 77, 1632 to give a mixture of acetates from which the title compound could be isolated.

- (3 β ,5 α ,11 α ,25R)-3,11-di(acetoxy)spirostan-12-one: (3 β ,5 α ,11 α ,25R)-3,11-di(acetoxy)spirostan-12-ol was oxidized with chromium trioxide and pyridine in methylene chloride according to the procedure described in Org. Syn., 1976, 55, 84 to give the title compound.

- 25 (3 β ,5 α ,11 α ,25R)-spirostan-3,11-diol-12-one: (3 β ,5 α ,11 α ,25R)-3,11-di(acetoxy)spirostan-12-one was saponified with sodium methoxide in methanol and THF to give the title compound.

Preparation BB10

(3 β ,5 α ,11 α ,12 α ,25R)-spirostan-3,11,12-triol

- 30 (3 β ,5 α ,25R)-spirostan-3-ol-12-tosylhydrazone: (3 β ,5 α ,25R)-spirostan-3-ol-12-one (8.00 g) was dissolved in glacial acetic acid (200 mL) and warmed to 50°C. Para-toluenesulfonylhydrazide (6.928 g) was added and the solution was stirred at 50°C for 30 min. After an additional 2 hours of stirring at room temperature, water

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(200 mL) was added. The resulting solid was collected, washed with water (100 mL), dried, triturated with refluxing acetone (300 mL), filtered hot and dried to give 3.903 g of the title compound.

- 5 (3 β ,5 α ,25R)spirost-11-en-3-ol: A mixture of (3 β ,5 α ,25R)spirostan-3-ol-12-tosylhydrazone (9.100 g) and sodium methoxide (8.379 g) in DMF (200 mL) was heated to 150°C for 35 minutes, then cooled to room temperature. The mixture was then poured into ice water (1200 mL) and the resulting suspension filtered. The collected solid was washed with water (100 mL), air-dried, and dissolved in
- 10 methylene chloride (700 mL). This solution was washed with water (2 X 200 mL), dried with MgSO₄, and concentrated to give a white solid. Following flash chromatography, 2.384 g of the title compound (mp 179-181°C, lit. 188-192°C - J. Am. Chem. Soc., 1954, 76, 4013) was isolated.

- (3 β ,5 α ,11 α ,12 α ,25R)spirostan-3,11,12-triol: (3 β ,5 α ,25R)spirost-11-en-3-ol was
- 15 oxidized to the title compound with osmium tetroxide and N-methylmorpholine-N-oxide in water, t-butanol and acetone according to the procedure describe in Tetrahedron Letters, 1976, 1973.

Preparation BB11

(3 β ,5 α ,12 β ,25R)spirostan-3,12-diol-11-one

- 20 (3 β ,5 α ,11 β ,25R)-11-bromospirostan-3-ol-12-one: A glass lined reactor was charged with 50 gallons of methanol then subsurface sparged with hydrochloric acid gas until 7.7 Kg (5.0 eq) were charged. Upon completion of this sparge, the reactor was charged with 18.8 Kg (42.2 mole) of (3 β ,5 α ,25R)spirostan-3-ol-12-one, 50 gallons of methanol and 10 gallons of methylene chloride. This mixture was cooled
- 25 to 10 °C and a solution of 8.4 Kg bromine (52.7 mole, 1.25 eq) in 10 gallons of methylene chloride was added over 2 hours while a pot temperature of approximately 10°C was maintained. Once the addition was complete the reaction was allowed to warm to room temperature and was stirred for 2 hours. TLC at this point indicated complete reaction.
- 30 The reaction was diluted with 50 gallons of water and stirred for 10 minutes. After separation of layers, the aqueous layer was extracted twice with 30 gallons of methylene chloride. The three combined organic extracts were washed twice with 30 gallons of water, once with 30 gallons of saturated brine, then dried using 7.0 Kg

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of magnesium sulfate. The drying agent was removed by filtration on a 30 inch Lapp followed by two 3 gallon methylene chloride washes. The filtrate and washes were combined and atmospherically distilled to a 7 gallon total volume. Two 10 gallon methanol charges were made followed by continued distillation. When a final volume of <10 gallons had been reached the mixture was cooled to room temperature. The resulting suspension was granulated for 2 hours, filtered on a 30 inch Lapp, and the filter cake was washed twice with 3 gallons of methanol. Vacuum drying the filter cake at 45-50° C yielded 12.6 Kg (58.6% yield) of the title compound.

- 10 (3 β ,5 α ,12 β ,25R)spirostan-3,12-diol-11-one: A glass lined reactor was charged with 12.4 Kg of (3 β ,5 α ,11 β ,25R)-11-bromospirostan-3-ol-12-one (24.34 mole), 33 gallons of t-butanol, 33 gallons of water and 7.5 Kg (189 mole, 7.75 eq) of sodium hydroxide pellets. The reaction was heated to reflux over 1.5 hours, maintained at reflux for 4.5 hours (pot temperature was 83° C), then cooled to room temperature.
- 15 TLC at this point indicated complete reaction.

The reaction was distilled to remove the t-butanol. This was accomplished both by vacuum and atmospheric distillation. During the concentration two 32.5 gallon charges of water were added. Once the t-butanol had been removed, the aqueous suspension was cooled to room temperature and granulated for 2 hours.

- 20 The suspension was filtered on a 30 inch Lapp, washed twice with 3 gallons of water, and the filter cake was air dried at 60 °C. This afforded 11.1 Kg of the title compound.

Preparation BB12

(3 β ,5 α ,25R)spirostan-3-ol-11-one

- 25 (3 β ,5 α ,12 β ,25R)-3,12-diacetoxyspirostan-11-one: A glass lined reactor was charged with 26 gallons of pyridine, 26 gallons of acetic anhydride and 11.0 Kg of (3 β ,5 α ,12 β ,25R)spirostan-3,12-diol-11-one (preparation G12). This mixture was refluxed for 2 hours (pot temperature 128° C) and allowed to cool to room temperature. The reaction was vacuum distilled to a total volume of 15 gallons (pot temperature approximately 45° C during distillation). The suspension was diluted with 25 gallons of acetic acid and further vacuum distilled to a 15 gallon total volume (pot temperature at end approximately 80° C). The mixture was diluted with 87 gallons of water and cooled to room temperature. After 5 hours of granulation, the
- 30

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title compound was isolated by filtration on a 30 inch Lapp followed by two 3 gallon water washes. The filter cake was dried at 60° C under vacuum to yield 12.2 Kg (93.3%).

(3 β ,5 α ,25R)spirostan-3-ol-11-one: A stainless steel reactor was cooled to
5 -80° C by passing liquid nitrogen through internal coils. Ammonia was added to the reactor until 54.5 Kg (80 liters, 3,200 mole, 170 eq) had been charged.

At the same time that the ammonia charge was commencing, a glass lined reactor was charged with 10.0 Kg of (3 β ,5 α ,12 β ,25R)-3,12-diacetoxyspirostan-11-one (18.84 mole) and 40 gallons of THF. This solution was atmospherically distilled until
10 a 26 gallon total volume had been reached.

At the completion of the ammonia charge, 2.8 Kg of calcium turnings (69.0 gram atoms, 3.7 eq) were added over 30 minutes while maintaining a pot temperature of -50° C. At the completion of this addition the THF solution of (3 β ,5 α ,12 β ,25R)-3,12-diacetoxyspirostan-11-one was added over 20 minutes (pot
15 temperature at the end of the addition was -35° C) followed by a 1.0 gallon THF rinse. The reaction mixture was stirred for 30 minutes at -35° C to -40° C. While the reaction was at -35° C to -40° C, 3.33 liters of bromobenzene (4.98 Kg, 31.7 mole, 1.68 eq) were added followed by 3.33 liters of water.

After this addition, the distillation of ammonia from the reactor was initiated.
20 This distillation was directed to a water scrubber. Once all of the ammonia had been removed, the reaction (now at 24° C) was transferred to a glass lined reactor followed by a 4 gallon THF rinse. The solution and rinse combined were vacuum distilled to a thick oil. To this was added 35 gallons of methanol and 3.3 Kg (59 mole) of potassium hydroxide pellets. This mixture was heated at reflux for 1 hour,
25 cooled, then 10 liters of acetic acid and 44 gallons of water were charged. This suspension was further cooled to room temperature and granulated for 1 hour. The title compound was isolated by filtration on a 30 inch Lapp followed by a 5 gallon 3:1 water/methanol wash. Vacuum drying at 55° C yielded 7.05 Kg (86.9%).

Preparation BB: Physical Data

30 Satisfactory MS and IR data was obtained on all of the (3 β ,5 α ,25R)spirostan-3-ols described in the above BB preparations (see table 2). The various diol and triol products could be distinguished by proton NMR (see table 3)

Table 2: Diagnostic Mass Spectrometry and Infrared Data

LSMIS IR			
	molecular	parent ion	diagnostic resonances
<u>compound</u>	<u>formula</u>	<u>(m/z)</u>	<u>(cm-1, intensity, solvent)</u>
5 11 α -ol	C ₂₇ H ₄₄ O ₄	433	3575 (m), 3440 (m) (CHCl ₃)
11 β -ol	C ₂₇ H ₄₄ O ₄	433	3560 (m), 3425 (m) (CHCl ₃)
12 α -ol	C ₂₇ H ₄₄ O ₄	433	3590 (m), 3420 (m) (CHCl ₃)
12 β -ol	C ₂₇ H ₄₄ O ₄	433	—
11 α ,12 α -diol	C ₂₇ H ₄₄ O ₅	449	3424 (m) (KBr)
10 11 α ,12 β -diol	C ₂₇ H ₄₄ O ₅	449	3550 (m), 3450 (m) (CHCl ₃)
11 β ,12 α -diol	C ₂₇ H ₄₄ O ₅	449	3441 (m) (KBr)
11 β ,12 β -diol	C ₂₇ H ₄₄ O ₅	449	3600 (m), 3450 (m) (CHCl ₃)
11 α -ol-12-one	C ₂₇ H ₄₂ O ₅	447	3515 (m), 1705 (s) (KBr)
11 β -ol-12-one	C ₂₇ H ₄₂ O ₅	447	3450 (m), 1712 (s) (KBr)
15 12 α -ol-11-one	C ₂₇ H ₄₂ O ₅	447	3410 (m), 1706 (s) (KBr)
12 β -ol-11-one	C ₂₇ H ₄₂ O ₅	447	3475 (m), 1708 (s) (CHCl ₃)
11,12-dione	C ₂₇ H ₄₀ O ₅	445	3600 (w), 3400 (m), 1710 (w) 1670 (s), 1605 (m) (CHCl ₃) ¹
11-one	C ₂₇ H ₄₂ O ₄	431	3600 (w), 3450 (m), 1705 (s) (CHCl ₃)

20

1 - IR data suggest that this compound readily tautomerizes enol ketone form in CHCl₃.

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Tabl 3: Diagnostic Proton Nuclear Magnetic Resonance Data²

<u>compound</u>	<u>peaks >2 ppm</u>
11 α -ol	3.90 (ddd, 6,6 & 4 Hz, 1H), 2.26 (dt, 13&4, 1H)
11 β -ol	4.22 (br s, 1 H)
5 12 α -ol	3.67 (s, 1 H), 2.37 (dd, 8 & 7 Hz, 1H)
12 β -ol	3.26 (dd, 10 & 4 Hz, 1H)
11 α ,12 α -diol	3.91(m, 1H), 3.56 (d, 3H, 1H), 2.45 (dd, 9 & 7 Hz, 1H)
11 α ,12 β -diol	3.55(m, 1H), 3.03 (d, 8H, 1H), 2.21 (dt,12 & 4 Hz, 1H)
10 11 β ,12 α -diol	4.12 (br s, 1H), 3.55 (d, 2 Hz, 1H), 2.36 (dd, 9 & 7 Hz, 1H)
11 β ,12 β -diol	4.07 (br s, 1H), 3.13 (d, 3 Hz, 1H)
11 α -ol-12-one	3.72 (m, 1H), 2.39 (dt, 13 & 4 Hz, 1H)
11 β -ol-12-one	3.96 (m, 1H), 2.2 (m, 1H)
15 12 α -ol-11-one	3.51 (s, 1H), 2.57 (dd, 8 & 7 Hz, 1H), 2.2 (complex, 7H)
12 β -ol-11-one	3.78 (s, 1H), 2.39 (dt,13 & 4 Hz, 1H), 2.1 (m, 2H)

2 - All samples run in CDCl₃ except 11 β -ol-12-one which was run in DMSO-d₆.

Peaks for H₁₆, H₃, H_{26eq} and H_{26ax} are also observed at >2 ppm. In CDCl₃, these are
 20 observed at 4.37 (ddd, J = 9, 9 & 7 Hz, 1H), 3.56 (heptet, J = 4 Hz, 1H), 3.45 (ddd, J = 10, 6 & 2 Hz, 1H), 3.35 (t, J = 11 Hz, 1H).

Preparation CC15-Methyl-2-thienyl-methylamine

Methoxyamine hydrochloride (3.5 g, 41.7 mmol) and pyridine (6.75 mL, 83.5
 25 mmol) was added to a solution of 5-methylthiophene-2-carboxaldehyde (3.5 g, 27.8 mmol) in methanol (20 mL) at room temperature. After 3h, the mixture was concentrated in vacuo, diluted with ethyl acetate (100 mL) and washed with 1N HCl (3x) and brine (1x), dried (Na₂SO₄), filtered and concentrated in vacuo to give a mixture of oxime isomers.

30 Trifluoroacetic acid (4.97 mL, 65 mmol) was added dropwise to a suspension of sodium borohydride (2.44 g, 65 mmol) in THF (40 mL) at 0°C. A solution of the oxime prepared above (2.0g, 13 mmol) in THF (5 mL) was then added dropwise. After 2 h, the reaction was heated to a gentle reflux for 2h, cooled, and quenched by

-85-

the addition of water (10 mL). The mixture was diluted with methylene chloride (50 mL) and washed with brine (2x) and dried (MgSO₄) filtered and concentrated in vacuo. The residue was dissolved in 100 mL of diethyl ether and treated with saturated HCl in ether (3 mL). The precipitate was collected by vacuum filtration and washed with ether and dried to afford the titled amine as a white solid as its hydrochloride salt (1.2 g). ¹H NMR (250 MHz, d6 DMSO) δ 8.5 (bs, 3H); 7.0 (d, 1H, J = 2.0 Hz); 6.7 (m, 1H); 4.1 (bs, 2H); 2.4 (s, 3H).

In an analogous manner, the following compounds, Preparation CC2-CC3 were prepared from the appropriate starting material using the above general procedure.

Preparation CC2

5-Bromo-2-thienyl-methylamine

Preparation CC3

4-Bromothiophenyl-methylamine

Preparation DD

3-thienyl-methylamine hydrochloride

3-Thiophenecarbonitrile (2.0 g, 18.3 mmol) was added dropwise as a solution in THF (20 mL) to a suspension of lithium aluminum hydride (695 mg, 18.3 mmol) in THF (20 mL) at 0°C. After 1h at room temperature, the reaction was quenched by the sequential addition of H₂O (700 μ L), 15 % NaOH (700 μ L) and H₂O (2.1 mL). The mixture was diluted with ether (50mL) and dried (MgSO₄) filtered and concentrated in vacuo. The residue was dissolved in ether (50 mL) and treated with saturated HCl/ether (3 mL). The resulting precipitate was filtered and washed with ether and dried to afford the title compound as an off-white solid. ¹H NMR (250 MHz, d6 DMSO) δ 8.5 (bs, 3H); 7.65 (bs, 1H); 7.52 (dd, 1H, J = 6.0, 2.0 Hz); 7.3 (dd, 1H, J = 6.0 Hz); 4.0 (d, 2H, J = 6.0 Hz).

Preparation EE

α -(2-thienyl)-2-yl- α -trimethylsiloxy-acetonitrile

CYANOHYDRIN FORMATION

Zinc iodide (5 mg) was added to a solution of trimethylsilyl cyanide (3.27 mL, 0.025 M) and 2-thiophenecarbaldehyde (2.08 mL, 0.022 mol) in dichloromethane

-86-

(50 mL) at room temperature. After 24 h, the mixture was concentrated and used without purification. ^1H NMR (250 MHz, CDCl_3) δ 7.35(dd, 1H, $J = 5.0, 1.0$ Hz); 7.2 (dd, 1H, $J = 4.0, 1.0$ Hz); 7.0 (dd, 1H, $J = 5.0, 4.0$ Hz); 5.2 (s, 1H); 0.2 (s, 9H).

Preparation FF1

5

2-amino-1-(2-thienyl)-ethanol

NITRILE REDUCTION

A solution of α -(2-thienyl)- α -trimethylsiloxy-acetonitrile (4.77 g, 0.024 mol) in THF (10 mL) was added dropwise to a suspension of lithium aluminum hydride (1.0 g, 0.027 mol) in THF (20 mL) at 0°C . After 1.5 h at room temperature, the reaction was quenched by the sequential addition of H_2O (1 mL), 15% NaOH (1 mL) and H_2O (3 mL). The mixture was diluted with ether (50 mL), dried (MgSO_4), filtered and concentrated to give 2.7 g product as an oil. ^1H NMR (250 MHz, CDCl_3) δ 7.2 (m, 1H); 7.0 (m, 2H); 4.9 (m, 1H); 3.0 (ddd, 1H, $J = 15.0, 12.0, 5.0$ Hz); 2.9 (ddd, 1H, $J = 12.0, 5.0, 2.0$ Hz); 2.0 (bs, 2H).

15

Preparation GG1

Thiazol-2-yl-methylamine

AZIDE REDUCTION

Triphenylphosphine (2.08 g, 7.9 mmol) was added to a solution of 2-azidomethyl-thiazole (1.11 g, 7.9 mmol) in THF (20 mL). After 1 h, H_2O (214 μL) and ammonium hydroxide sol (0.5 mL) were added sequentially. The reaction stirred overnight, was concentrated in vacuo, and purified by flash chromatography (5 % methanol/methylene chloride) to afford 645 mg of the title product as a tan oil (72%). ^1H NMR (250 MHz, CDCl_3) δ 7.7 (d, 1H, $J = 2.0$ Hz); 7.25 (d, 1H, $J = 2.0$ Hz); 4.2 (s, 2H); 1.7 (bs, 2H).

25

In an analogous manner, the following compound, Preparation GG2 was prepared from the appropriate starting material using the above general procedure.

Preparation GG2

3-Methylisoxazol-5-yl-methylamine

30

Preparation HH1

2-azidomethyl-thiazole

Diphenylphosphoryl azide (3.25 mL, 0.015 mol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (2.25 mL, 0.025 mol) were added to a solution of

-87-

thiazol-2-yl-methanol (1.44 g, 0.013 mol) in toluene (20 mL) at 0°C. After 1 h the reaction was warmed to room temperature and stirred overnight. The mixture was diluted with toluene (20 mL) and washed with H₂O (3x) brine (1x), dried (Na₂SO₄), filtered and concentrated in vacuo. The residue was purified by flash

5 chromatography (30 % ethyl acetate/hexanes) to afford the azide as a tan oil (1.1g, 63%). IR 2098 cm⁻¹. ¹H NMR (250 MHz, CDCl₃) δ 7.8 (d, 1H, J = 2.0 Hz); 7.4 (d, 1H, J = 2.0 Hz); 4.7 (s, 2H).

In an analogous manner, the following compound, Preparation HH2 was prepared from the appropriate starting material using the above general procedure.

10

Preparation HH2

5-azidomethyl-3methylisoxazole

Preparation II1:

3-amino-2,2-dimethyl-propionic acid methyl ester

15

Raney nickel (2g) was washed with water then methanol and added to a solution of methyl 2,2-dimethyl cycloacetate (2.0 g, 0.016 mol) in methanol (75 mL). The mixture was shaken under 40 psi of hydrogen in a Parr apparatus. After 3 h, the reaction was purged with nitrogen. The catalyst was removed by filtration and the filtrate was acidified by the addition of sat HCl/ether (5 mL). The solution was concentrated to approx. 5 mL and added to 200 mL of vigorously stirred ether. The resulting solid was collected by vacuum filtration, washed with ether and dried to afford 1.25 g of amine hydrochloride as a white solid. m.p. 168-170°C. ¹H NMR (250 MHz, CDCl₃) δ 8.3 (bs, 3H); 3.6 (s, 3H); 2.92 (m, 2H); 1.2 (s, 6H).

25

In an analogous manner, the following compound, Preparation II2 was prepared from the appropriate starting material using the above general procedure.

Preparation II2

2-aminomethyl-butyric acid methyl ester

30

Preparation JJ

2-thienyl isocyanate

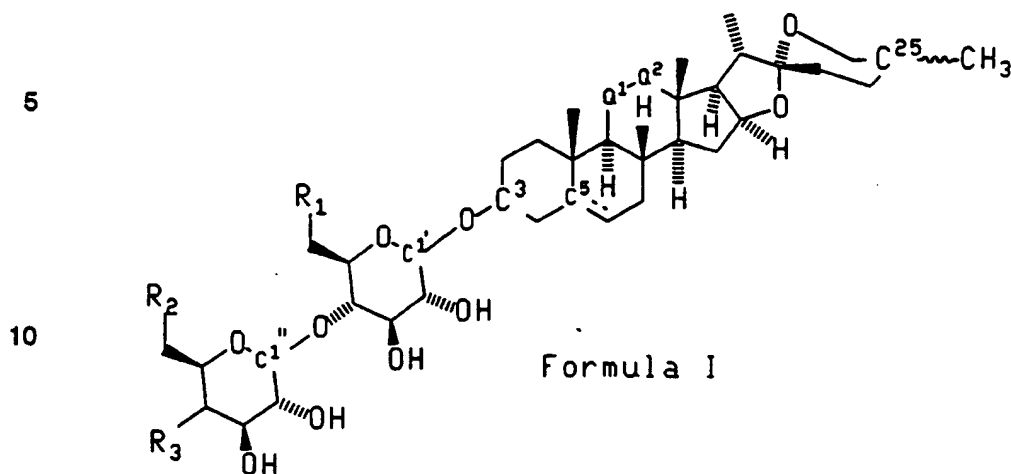
Sodium azide (2.5 g, 0.039 mol) as a solution in H₂O (10 mL) was added dropwise to a solution of 2-thiophenecarbonyl chloride (5.7 g, 0.039 mol) in toluene

-88-

(80 mL) at 0°C. After 30 min the layers were separated and the aqueous layer extracted with ether. The combined organic layers were dried (MgSO_4), filtered and the ether was removed in vacuo. The solution of 2-thiophenecarbonyl azide was heated at 85°C for 3h producing a solution of 2-thienyl-isocyanate which was used
5 as is.

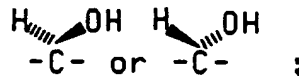
CLAIMS

1. A spirostanyl glycoside compound of Formula I



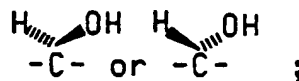
15 and the pharmaceutically-acceptable salts and hydrates thereof
wherein

Q' is carbonyl,



20

Q² is methylene, carbonyl,



25 R₁, R₂, and R₃ are each hydrogen, hydroxy, halo, amino, azido, (C₁-C₆)alkoxy(C₁-C₆)alkoxy or -Z-R₄;

Z for each occurrence is independently -NH-C(=O)-, -O-C(=O)-, -O-C(=O)-N(R⁵)-, -NH-C(=O)-N(R⁵)- or -O-C(=S)-N(R⁵)-;

R_4 for each occurrence is independently aryl, aryl(C₁-C₆)alkyl, (C₂-C₄)alkenyl, (C₁-C₆)alkyl, cyclo(C₃-C₇)alkyl or cyclo(C₃-C₇)alkyl(C₁-C₆)alkyl; each R_4 optionally mono-, di-, or tri-substituted independently with halo, (C₁-C₄)alkyl, hydroxy, phenoxy, trifluoromethyl, nitro, (C₁-C₄)alkoxy, methylenedioxy, oxo, (C₁-C₄)alkylsulfanyl, (C₁-C₄)alkylsulfinyl, (C₁-C₄)alkylsulfonyl, dimethylamino, mono- or di-(C₁-

-90-


C₄)alkylaminocarbonyl, (C₁-C₄)alkylcarbonyl, (C₁-C₄)alkoxycarbonyl, pyrrolidinylcarbonyl wherein aryl is carbon-linked and is phenyl, furyl, thienyl, pyrrolyl, oxazolyl, isoxazolyl, oxadiazolyl, thiazolyl, isothiazolyl, benzothiazolyl, thiadiazolyl, pyrazolyl, imidazolyl or pyridyl; and

- 5 R₅ for each occurrence is independently hydrogen, (C₁-C₄)alkyl or R₅ is such that when taken together with the nitrogen to which it is attached and with R₄, wherein R₄ is a covalent bond, it forms pyrrolidinyl, piperidinyl, N-methylpiperazinyl, indolinyl or morpholinyl and such cyclic group may be substituted on carbon with (C₁-C₄)alkoxycarbonyl;
- 10 with the proviso that R₁, R₂ and R₃ are not all hydroxy.
2. A compound according to claim 1 wherein R₁, R₂ and R₃ are each independently hydroxy or -Z-R₄, Z is -O-C(=O)-N(R₅)- and R₅ is hydrogen.
3. A compound according to claim 2 wherein the C^{1'} anomeric oxy is beta, the C^{1'} anomeric oxy is beta, R₃ is alpha, the C⁵ hydrogen is alpha, C²⁵ is (R), the
- 15 C³ oxy is beta, Q¹ is carbonyl, Q² is methylene and R₁ is hydroxy.
4. A compound according to claim 3 wherein R₃ is hydroxy, R₂ is -Z-R₄ and R₄ is 2,4-difluorophenyl.
5. A compound according to claim 3 where R₂ and R₃ are -Z-R₄ and R₄ is 2-fluorophenyl.
- 20 6. A compound according to claim 3 where R₂ and R₃ are -Z-R₄ and R₄ is 2,4-difluorophenyl.
7. A compound according to claim 3 wherein R₂ and R₃ are -Z-R₄ and R₄ is 2-methylphenyl.
8. A compound according to claim 3 where R₂ and R₃ are -Z-R₄ and R₄ is
- 25 phenyl.
9. A compound according to claim 3 wherein R₂ is -Z-R₄, R₄ is 2,6-dichlorophenyl and R₃ is hydroxy.
10. A compound according to claim 3 wherein R₂ is -Z-R₄, R₄ is 2-fluorophenyl and R₃ is hydroxy.
- 30 11. A compound according to claim 3 wherein R₂ and R₃ are Z-R₄ and R₄ is 2-thienyl-methyl.
12. A compound according to claim 3 wherein R₂ and R₃ are Z-R₄ and R₄ is 2-methoxycarbonyl-ethyl.

13. A compound according to claim 3 wherein R_2 and R_3 are $Z-R_4$ and R_4 is thiazol-2-yl-methyl.

14. A compound according to claim 3 wherein R_2 and R_3 are $Z-R_4$ and R_4 is 2-methoxycarbonyl-butyl.

5 15. A compound according to claim 2 wherein the $C^{1'}$ anomeric oxy is beta, the $C^{1''}$ anomeric oxy is beta, R_3 is alpha, the C^5 hydrogen is alpha, C^{25} is (R), the C^3

oxy is beta, Q^1 is carbonyl, Q^2 is  and R_1 is hydroxy.

16. A compound according to claim 15 wherein R_2 and R_3 are $Z-R_4$ and R_4 is 2-fluorophenyl.

10 17. A compound according to claim 15 wherein R_2 and R_3 are $Z-R_4$ and R_4 is 2-thienyl-methyl.

18. A compound according to claim 15 wherein R_2 and R_3 are $Z-R_4$ and R_4 is 2-methoxycarbonyl-ethyl.

15 19. A compound according to claim 15 wherein R_2 and R_3 are $Z-R_4$ and R_4 is thiazol-2-yl-methyl.

20. A compound according to claim 1 wherein Q^1 is carbonyl, R_1 is hydroxy, hydrogen, halo, azido, or (C_1-C_6) alkoxy (C_1-C_6) alkoxy, R_2 is hydrogen, halo, azido, or (C_1-C_6) alkoxy (C_1-C_6) alkoxy, R_3 is $Z-R_4$, Z is $-O-C(=O)N(R_5)-$ and R_5 is hydrogen.

20 21. A compound according to claim 20 wherein the $C^{1'}$ anomeric oxy is beta, the $C^{1''}$ anomeric oxy is beta, R_3 is alpha, the C^5 hydrogen is alpha, C^{25} is (R) and the C^3 oxy is beta.

22. A compound according to claim 21 wherein R_1 is hydroxy, R_2 is hydrogen and R_4 is 2-fluorophenyl.

25 23. A compound according to claim 1 wherein R_1 , R_2 and R_3 are each independently hydroxy or $-Z-R_4$, Z is $-O-C(=O)-$, R_4 is independently (C_1-C_6) alkyl, aryl or aryl mono- or disubstituted with halo or (C_1-C_6) alkyl.

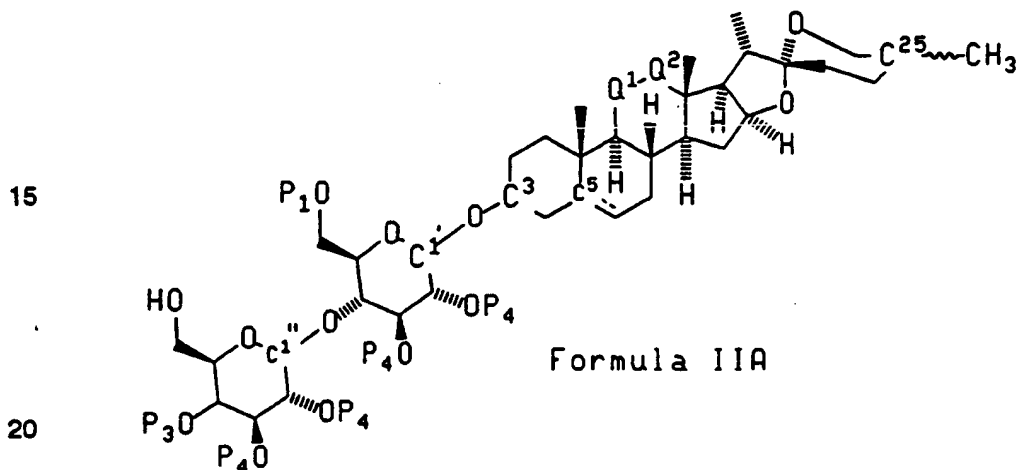
30 24. A compound according to claim 23 wherein R_1 and R_2 are $-Z-R_4$, R_3 is alpha hydroxy, R_4 is furan-2-yl, the $C^{1'}$ anomeric oxy is beta, the $C^{1''}$ anomeric oxy is beta, the C^5 hydrogen is alpha, C^{25} is (R), the C^3 oxy is beta, Q^1 is carbonyl and Q^2 is methylene.

25. A compound according to claim 1 wherein R₁, R₂ and R₃ are each independently hydroxy or halo, Q' is carbonyl, the C' anomeric oxy is beta and R₃ is alpha.

26. A method of treating hypercholesterolemia which comprises
5 administering to a mammal in need of such treatment a therapeutically effective
amount of a compound of claim 1.

27. A pharmaceutical composition for the treatment of hypercholesterolemia in a mammal which comprises a therapeutically effective amount of compound of claim 1 and a pharmaceutically acceptable carrier.

10 28. A spirostanyl glycoside compound of Formula II A

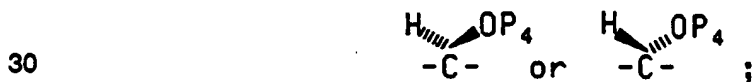


wherein

Q' is carbonyl,



Q² is methylene, carbonyl,



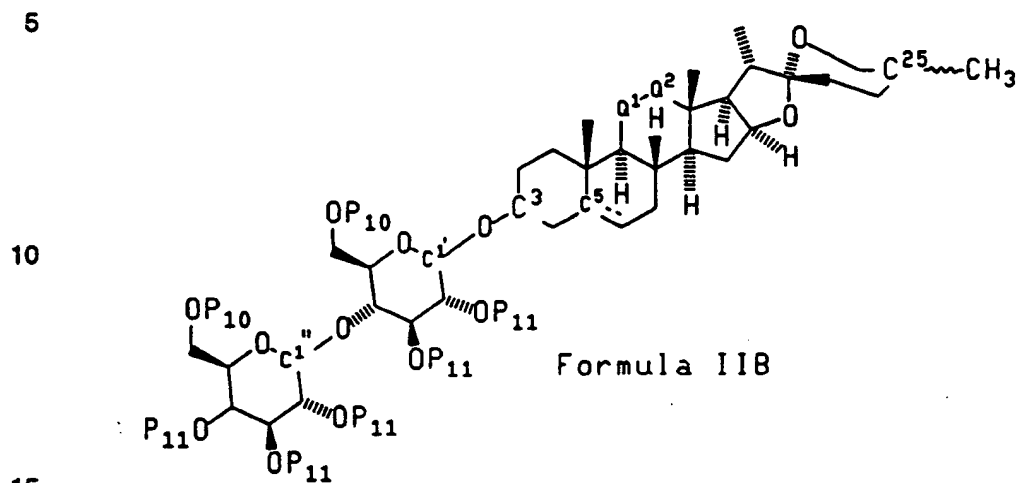
P₄ is an alcohol protecting group; and

P₁ is hydrogen and P₂ is an alcohol protecting group or

P₃ is hydrogen and P₁ is an alcohol protecting group.

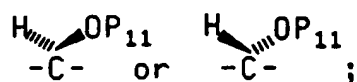
29. The compound as recited in claim 28 wherein the alcohol protecting group is acetyl or chloroacetyl.

30. A spirostanyl glycoside compound of Formula IIB

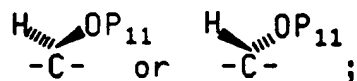


wherein

Q' is carbonyl,



Q² is methylene, carbonyl,



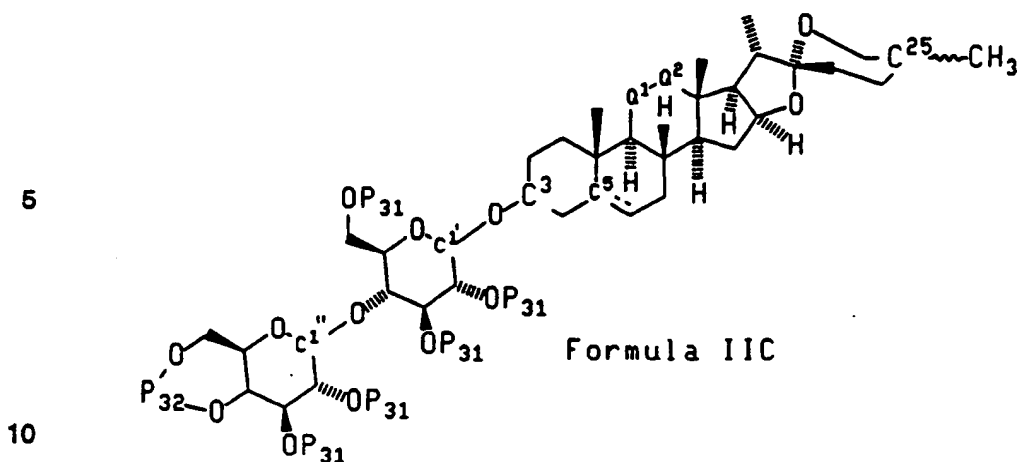
25 P_{10} is a silyl protecting group; and

P₁₁ is an alcohol protecting group that is different from P₁₀.

31. A compound as recited in claim 30 wherein the alcohol protecting group is acetyl and the silyl protecting group is t-butyl diphenylsilyl, triisopropylsilyl or t-butyl dimethylsilyl.

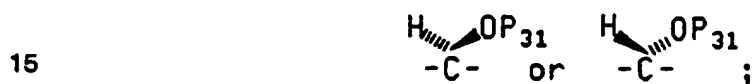
30 32. A spirostanyl glycoside compound of Formula IIC

-94-



wherein

Q¹ is carbonyl,



Q² is methylene, carbonyl,



P₃₁ is an alcohol protecting group, and

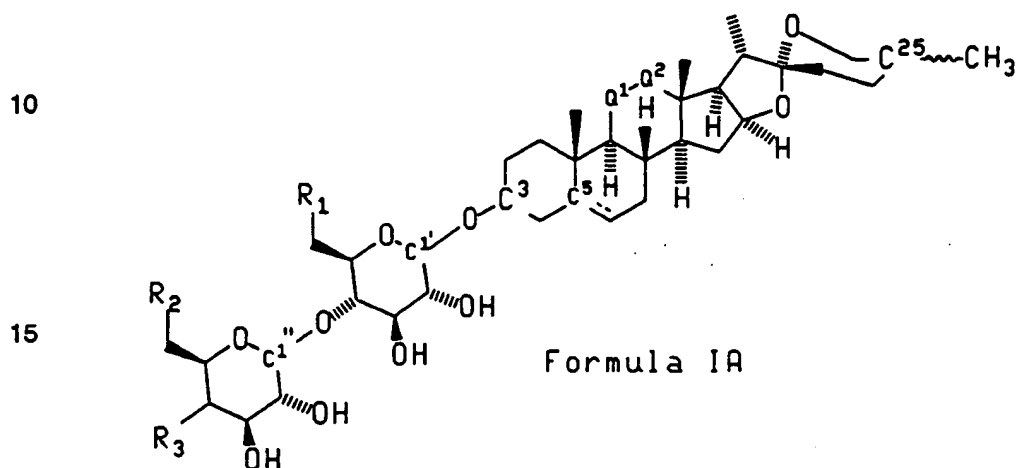
P₃₂ forms a cyclic protecting group for a 1,3 diol.

33. A compound as recited in claim 32 wherein P₃₁ is acetyl or chloroacetyl and P₃₂ is benzylidene or paramethoxybenzylidene.

34. A method of treating atherosclerosis which comprises administering to a mammal in need of such treatment a therapeutically effective amount of a compound of claim 1.

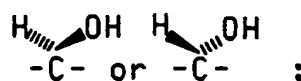
35. A pharmaceutical composition for the treatment of atherosclerosis in a mammal which comprises a therapeutically effective amount of compound of claim 1 and a pharmaceutically acceptable carrier.

36. A process for preparing a compound of Formula IA

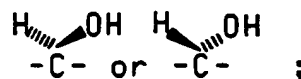


20 and the pharmaceutically-acceptable salts and hydrates thereof wherein

Q¹ is carbonyl,



Q² is methylene, carbonyl,

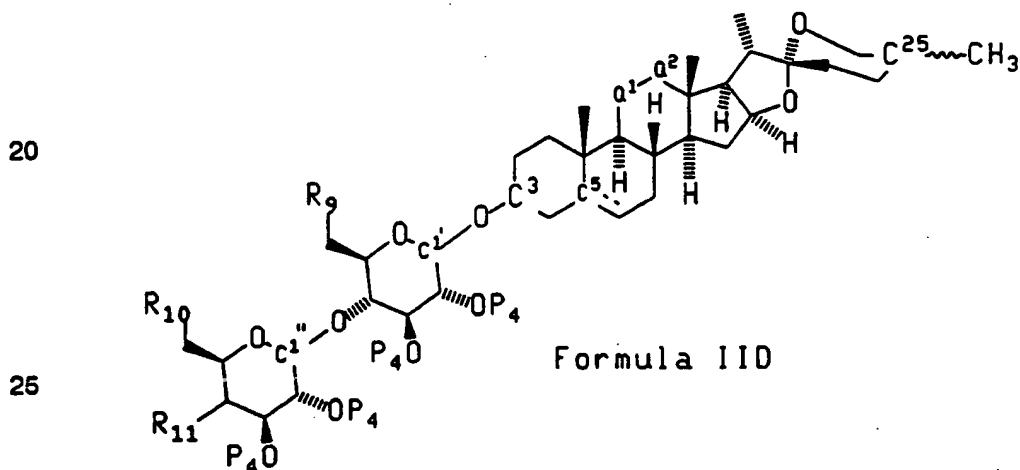


30 R₁, R₂ and R₃ are each independently hydrogen, hydroxy, halo, amino, azido, (C₁-C₆)alkoxy(C₁-C₆)alkoxy or -Z-R₄;

Z for each occurrence is independently -NH-C(=O)-, -O-C(=O)-, -O-C(=O)-N(R⁵)-, -NH-C(=O)-N(R⁵)- or -O-C(=S)-N(R⁵)-;

-96-

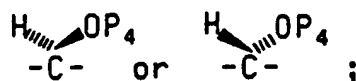
- R_4 for each occurrence is independently aryl, aryl(C₁-C₆)alkyl, (C₂-C₄)alkenyl, (C₁-C₆)alkyl, cyclo(C₃-C₇)alkyl or cyclo(C₃-C₇)alkyl(C₁-C₆)alkyl; each R_4 optionally mono-, di-, or tri-substituted independently with halo, (C₁-C₄)alkyl, hydroxy, phenoxy, trifluoromethyl, nitro, (C₁-C₄)alkoxy, methylenedioxy, oxo, (C₁-C₄)alkylsulfanyl, (C₁-C₄)alkylsulfinyl, (C₁-C₄)alkylsulfonyl, dimethylamino, mono- or di-(C₁-C₄)alkylaminocarbonyl, (C₁-C₄)alkylcarbonyl, (C₁-C₄)alkoxycarbonyl, pyrrolidinylcarbonyl wherein aryl is carbon-linked and is phenyl, furyl, thienyl, pyrrolyl, oxazolyl, isoxazolyl, oxadiazolyl, thiazolyl, isothiazolyl, benzothiazolyl, thiadiazolyl, pyrazolyl, imidazolyl or pyridyl; and
- R_5 for each occurrence is independently hydrogen, (C₁-C₄)alkyl or R_5 is such that when taken together with the nitrogen to which it is attached and with R_4 , wherein R_4 is a covalent bond, it forms pyrrolidinyl, piperidinyl, N-methylpiperazinyl, indolyl or morpholyl and such cyclic group may be substituted on carbon with (C₁-C₄)alkoxycarbonyl;
- with the proviso that R_1 , R_2 and R_3 are not all hydroxy which process comprises deprotecting a compound of Formula IID



wherein

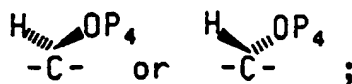
 Q' is carbonyl,

30



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Q^2 is methylene, carbonyl,



5 P_4 is an alcohol protecting group;

R_9 , R_{10} and R_{11} are each independently, hydrogen, hydroxy, halo, amino, azido, (C_1-C_6) alkoxy (C_1-C_6) alkoxy, $-Z-R_4$ or an alcohol protecting group attached through an oxy;

Z for each occurrence is independently $-\text{NH}-\text{C}(=\text{O})-$, $-\text{O}-\text{C}(=\text{O})-$, $-\text{O}-\text{C}(=\text{O})-$

10 $\text{N}(\text{R}^5)-$, $-\text{NH}-\text{C}(=\text{O})-\text{N}(\text{R}^5)-$ or $-\text{O}-\text{C}(=\text{S})-\text{N}(\text{R}^5)-$;

R_4 for each occurrence is independently aryl, aryl (C_1-C_6) alkyl, (C_2-C_4) alkenyl, (C_1-C_6) alkyl, cyclo (C_3-C_7) alkyl or cyclo (C_3-C_7) alkyl (C_1-C_6) alkyl; each R_4 optionally mono-, di-, or tri-substituted independently with halo, (C_1-C_4) alkyl, hydroxy, phenoxy, trifluoromethyl, nitro, (C_1-C_4) alkoxy, methylenedioxy, oxo, (C_1-C_4) alkylsulfanyl, $(C_1-$

15 $C_4)$ alkylsulfanyl, (C_1-C_4) alkylsulfonyl, dimethylamino, mono- or di- (C_1-C_4) alkylaminocarbonyl, (C_1-C_4) alkylcarbonyl, (C_1-C_4) alkoxycarbonyl, pyrrolidinylcarbonyl wherein aryl is carbon-linked and is phenyl, furyl, thienyl, pyrrolyl, oxazolyl, isoxazolyl, oxadiazolyl, thiazolyl, isothiazolyl, benzothiazolyl, thiadiazolyl, pyrazolyl, imidazolyl or pyridyl; and

20 R_5 for each occurrence is independently hydrogen, (C_1-C_4) alkyl or R_5 is such that when taken together with the nitrogen to which it is attached and with R_4 , wherein R_4 is a covalent bond, it forms pyrrolidinyl, piperidinyl, N-methylpiperazinyl, indolinyl or morpholinyl and such cyclic group may be substituted on carbon with (C_1-C_4) alkoxycarbonyl.

25 37. The process according to claim 36 wherein R_{10} and R_{11} are each $-\text{O}-\text{C}(=\text{O})-\text{N}(\text{R}_5)-R_4$, R_9 is an alcohol protecting group connected through an oxy, R_5 is hydrogen and the Formula IID compound is deprotected by reaction with a nucleophilic base in a polar solvent at temperatures of about 0°C to about 100°C .

38. The process according to claim 37 wherein the $C^{1'}$ anomeric oxy is beta,
30 the $C^{1'}$ anomeric oxy is beta, R_{11} is alpha, the C^5 hydrogen is alpha, C^{25} is (R), the

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C^3 oxy is beta, Q^1 is carbonyl, Q^2 methylene $\text{r} \begin{array}{c} \text{H} \diagup \text{OH} \\ | \\ -\text{C}- \end{array}$ and the alcohol protecting group is acetyl or chloroacetyl.

39. The process according to claim 38 wherein R_4 is 2,4-difluorophenyl, phenyl, 2-fluorophenyl, 2-methylphenyl, 2-thienyl-methyl, 2-methoxycarbonyl-ethyl, 5 thiazol-2-yl-methyl or 2-methoxycarbonyl-butyl.

A. CLASSIFICATION OF SUBJECT MATTER IPC 6 C07J71/00 A61K31/58		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) IPC 6 C07J A61K		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used)		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,93 11150 (PFIZER INC.) 10 June 1993 see page 4, line 15; examples 4,5	1,2, 26-29, 34-39
A	JOURNAL OF PHARMACEUTICAL SCIENCES, vol.67, no.11, 1978, WASHINGTON D.C., US pages 1589 - 1592 R. SEGAL ET AL 'Hemolytic properties of synthetic glycosides' see the whole document	1,26-28, 30,32, 34-36
A	JUSTUS LIEBIGS ANNALEN DER CHEMIE, vol.699, 1966, WEINHEIM DE pages 212 - 222 R. TSCHESCHE ET AL 'Über parillin, ein saponin mit stark verzweigter zuckerkette' see the whole document	1,26-28, 30,32, 34-36
-/--		
<input checked="" type="checkbox"/> Further documents are listed in the continuation of box C. <input checked="" type="checkbox"/> Patent family members are listed in annex.		
* Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "B" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. "&" document member of the same patent family		
Date of the actual completion of the international search 31 January 1995		Date of mailing of the international search report 08.02.95
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016		Authorized officer Moreno, C

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO,A,94 00480 (PFIZER INC.) 6 January 1994 see the whole document ---	1-39
P,X	WO,A,94 00478 (PFIZER INC.) 6 January 1994 see claim 9; examples -----	1,2, 26-29, 34-39

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IB 94/00348

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 26, 34
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 26 and 34 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9311150	10-06-93	AU-A- 2775892	28-06-93
		CA-A- 2123684	10-06-93
		EP-A- 0619822	19-10-94
		FI-A- 942394	24-05-94
		JP-T- 6510794	01-12-94
		NO-A- 941919	24-05-94
		PT-A- 101088	28-02-94

WO-A-9400480	06-01-94	AU-B- 4226593	24-01-94
		CN-A- 1085561	20-04-94

WO-A-9400478	06-01-94	NONE	
